

Exposure to benzyloperazine (BZP) in adolescent rats: Adulthood
changes in anxiety-like behaviour.

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By

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Abbreviations

ADHD	Attention deficit hyperactivity disorder
AMP	Amphetamine
BZP	Benzylpiperazine
CNS	Central nervous system
DA	Dopamine
DAT	Dopamine transporter
DOA	Drugs of abuse
DSM-IV	The Diagnostic and Statistical Manual of Mental Disorders
F	Female
GAD	Generalized anxiety disorder
i.p.	Intraperitoneal injection
M	Male
MDMA	Methylenedioxymethamphetamine (Ecstasy)
METH	Methamphetamine
Mg	Milligrams
mg/kg	Milligrams per kilogram
MPH	Methylphenidate (Ritalin)
N	Number
NAc	Nucleus Accumbens
OF	Open field
PET	Positron emission tomography
PFC	Pre frontal cortex
PND	Post natal day
S	Saline
S.E.M.	Standard error of the mean
SUD	Substance use disorder
TFMPP	Trifluoromethylphenylpiperazine
5-HT	Serotonin
5-HTT	Serotonin transporter

Abstract

Increasingly, individuals in New Zealand are taking “herbal highs” with little knowledge of their possible long-term effects. Benzylpiperazine (BZP) is the predominant base drug in most herbal highs. The limited research into BZP has suggested that it produces similar effects to amphetamine, but could be 10 times less potent. There are to date, however, no long-term behavioural studies of BZP exposure. This study therefore, investigated effects of BZP exposure in adolescent male and female rats on subsequent measures of anxiety-like behaviours in adulthood. One group of experimental animals was treated daily with BZP, whereas another group received the same total amount of drug via a four day “binge” regime. The results suggested that, when observed in a Y-maze, social interaction test and a light/dark emergence test, BZP-treated rats were more anxious than control animals. In the Y-maze, male controls were more active than female controls, but BZP-treated females were more active than treated males. Results of this interaction indicate that the male rats may have been more affected by the administration of BZP during adolescence than females. Additionally, rats given the binge dose regime showed significantly increased anxiety in the Y-maze relative to the daily-exposed or control rats’. This suggests that larger quantities of BZP over a shorter time frame produce more detrimental effects than smaller quantities of BZP over a longer time frame. Overall, it would appear that the administration of BZP to adolescent animals produces behavioural changes in emotionality that are detectable in adulthood.

1.0 Introduction

1.1 General Overview

Adolescence is a vulnerable period in an individual's life. Any choices or changes made to the developing body or brain during this time can impact positively or negatively on the functioning of the individual in adulthood. Extensive literature and research has addressed adulthood drug use and consequences of this use (Clemens, Van Nieuwenhuyzen, Li, Cornish, Hunt, & McGregor, 2004; Robinson & Kolb, 2004). There is, however, limited research into the long-term consequences of adolescent drug use on subsequent functioning in adulthood. Experimentation with drugs or mind-altering substances typically begins during adolescence (Merline, O'Malley, Schulenberg, Bachman, & Johnston, 2004). New Zealand health promotion strategies focus on harm reduction methods and an appreciation of adolescent psychosocial development in which experimentation with drugs plays a part (Bennett & Coggan, 2000). Harm reduction is based on the idea that mood-altering substances are a normal part of human nature and use of them should be reduced rather than totally banned. Benzylpiperazine (BZP) was initially introduced into New Zealand as part of the policy of harm reduction (Bowden, 2004). There is evidence from studies on humans (Bye, Munro-Faure, Peck, & Young, 1973; Campbell, Cline, Evans, Lloyd, & Peck, 1973; Wikstrom, Holmgren, & Ahlner, 2004), rodents (Baumann, Clark, Budzynski, Partilla, Blough, & Rothman, 2004; Oberlander, Euvrard, Claude, & Boisser, 1979) and monkeys (Fantegrossi, Winger, Woods, Woolverton, & Coop, 2005) that BZP has stimulant drug properties comparable to amphetamine-like drugs. Therefore, BZP, "party pills" or "herbal highs" are marketed as a safe and legal alternative (Janes, 2004) and aimed at the population of individuals abusing amphetamine (AMP) or methamphetamine (METH). There is, however, still no evidence to support this presumption. Conversely, if this viewpoint is correct, it fails to consider the adolescent population of individuals who have embraced the consumption of the so-called "legal highs" (*The Christchurch Press*, 3 November 2005; 15 November 2004). Adolescents may initiate drug use for a variety of reasons, but once a drug of abuse has been administered to the human brain, profound changes can occur. It is

important to use an animal model to investigate possible long-term behavioural changes, as any subsequent BZP effects can be isolated from confounding variables that are observed in adolescent human populations. The primary aim of this study, therefore, is to investigate possible long-term change in behaviour expressed in adulthood arising from adolescent administration of BZP.

1.2. Substance Abuse

To assist in the understanding of the increasing worldwide substance abuse problem (Everitt, Dickinson, & Robbins, 2001; Stansfield & Kirstein, 2005), the diagnosis of substance abuse and dependence is presented. The complex and controversial aetiology of substance abuse itself is briefly explored. Additionally, before individuals are diagnosed with a substance use disorder (SUD), they must make the transition from experimentation to abuse. Two contrasting theories help to clarify this transition. A broader construct of the “stage” theory of addiction sheds some light on the possible consequences of long-term BZP use. New Zealand data suggest that there is an escalation of stimulant use and this can be explained by the easy availability of drugs in the individuals’ environments. Finally, this section addresses the escalating prevalence of substance use among adolescents.

For individuals presenting with SUD, the different categories of dependence and abuse have to be clearly differentiated. *The Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) is the leading tool used to diagnose substance abuse and dependence disorders (APA, 1994). To meet a diagnosis of substance dependence, an individual must meet 3 or more of the 7 criteria for dependence during the previous 12 months. To be given a diagnosis of substance abuse, the symptoms must not have met the criteria for substance dependence. In the previous 12 months an individual must have experienced 1 or more of the 4 criteria described as abuse symptoms (APA, 1994 provides a full description). These criteria are supported by animal drug research. Rats given extended access to self administered cocaine showed three well-established symptoms of substance

dependence, that is: an escalation in drug use, continued drug seeking and an increased motivation to self-administer cocaine (Ferrario, Gorny, Crombag, Li, Kolb, & Robinson, 2005). Therefore, according to DSM-IV criteria, an individual who experiences 3 or more of the dependence symptoms during the previous 12 months is considered to be substance dependent. Individuals whose symptoms have not met substance dependence criteria but who have experienced at least one abuse symptom are considered substance abusers. Additionally, despite mounting evidence showing detrimental consequences of substance use, individuals still experiment with drugs of abuse (DOA). However, the question of why people use or experiment with substances is complex.

The aetiology of drug abuse is complicated because of interactions among many psychological, environmental and biological risk factors related to drug use (Crombag & Robinson, 2004; Gilvarry, 2000). Examples of internal psychological risk factors include underlying mood disorders (Armstrong and Costello, 2002), sensation seeking (Barnea, Teichman, & Rahau, 1992) or impulsivity (Jentsch & Taylor, 1999). Environmental risk factors are one or more of the following: use of the drug in a drug-associated environment; use of drug paraphernalia; individual expectations of the drug being used; and peer pressure or the expectations of the social circle to which the individual belongs (Crombag & Robinson, 2004). Examples of biological risk factors are genetic predispositions (Merikangas, et al., 1998) and differences in the structure or biochemistry of the brain (Carlson, 2001). As a result, simple answers to questions of “what causes drug abuse?” do not exist (Spooner, 1999, p454). What is known, however, is that all substances act on the brain mechanisms responsible for positive reinforcement (Carlson, 2001). There is ample literature describing the possible causes of drug abuse, but the analysis of conflicting theories is beyond the scope of this thesis (Catalano, Kosterman, Hawkins, Newcomb, & Abbott, 1996; Everitt, et al., 2001; Laviola, Adriani, Terranova, & Gerra, 1999; Petraitis, Flay, & Miller, 1995; Robinson & Berridge, 2001; Spooner, 1999). What is supported in the literature is that there are differences between individuals and within the same individual at different stages of development, because of

variations in psychological, environmental and genetic factors. However, it is clear that drug experimentation is a common characteristic of adolescent behaviour during the transition into adulthood.

Many studies have attempted to explain how drug experimentation can lead to dependence. Drug experimentation is perceived to be a natural aspect of human behaviour (Smith, 2003). A large number of individuals experiment with substances for variable periods of time, yet only a few go on to develop an addiction (Piazza & LeMoal, 1998). For example, it is suggested that only 15 to 17 per cent of individuals experimenting with drugs become substance dependent (Deroche-Gamonet, Belin, & Piazza, 2004). Two principal theoretical structures explain the transition from drug experimentation to SUD: The first is the individual-centred concept of addiction, namely, that drug abuse is a pre-existing pathological condition in which certain individuals are biologically predisposed to be vulnerable to the appetitive properties of drugs. For example, some individuals may have increased or sensitised corticosterone levels that induce stress, which in turn increases vulnerability to drugs via the enhancement of the dopamine reward pathways, resulting in drug dependence (Piazza & LeMoal, 1996). The second theoretical structure is the drug-centred concept of addiction, in which the changes are thought to result from drug use (for example, tolerance, sensitization and conditioning, see Robinson & Berridge, 2001 or Wolf, et al., 1998) and are primarily responsible for the transition from use to abuse (Ferrario, et al., 2005; Robinson & Berridge, 2001). The present study addresses this drug-centred concept of addiction, by suggesting that changes in the developing brain during adolescence can cause long-term changes in adult behaviour. Regardless of which view is correct, the stage theory of addiction raises an important issue that must be considered in relation to adolescents experimenting with BZP.

In the stage theory of addiction, the development of SUD is considered to follow a lawful progression from legal drugs to illegal drug use. The key predictions of this theory are that

individuals using illegal drugs will have previously used legal drugs, and that not all individuals who use legal drugs will move on to illegal drugs (MacCoun, 1998). The common developmental sequence of SUD generally starts with alcohol or tobacco, and then moves on through inhalants and marijuana to illicit drugs (Smith, 2003; Walker, Venner, Hill, Meyer, & Miller, 2004). These substances have been deemed “gateway” drugs (Fergusson & Horwood, 2000), because the taking of these substances may lead into use of harder drugs.

Support for the stage theory of addiction comes from the Christchurch Health and Development Study (CHDS). This is a 21 year follow up study of 990 New Zealand children born in mid-1977. The authors measured frequency of cannabis use and other illegal drugs in the cohort of individuals when they were between 15 and 21 years of age. They controlled for family, social, behavioural and educational backgrounds prior to the age of 15 and additionally controlled for differences in adolescent lifestyle variables. By the age of 21, nearly 70% of the individuals had used cannabis and 26% had used other illegal drugs. Astonishingly, cannabis in all but three of the cases preceded the use of illegal drugs. The authors concluded that cannabis in New Zealand may act as a gateway drug, supporting the stage theory of addiction (Fergusson & Horwood, 2000). Proponents of the stage theory of addiction conclude that there is a general progression from legal to illegal drugs.

BZP may also be considered a gateway drug, but unlike cannabis, it is legal. The implications of this may be extremely important as recent research (Wilkins, Girling, Sweetser, & Butler, 2005, unpublished observation) suggests that cannabis and METH are considered easy to obtain in New Zealand, and it is extremely likely that users of BZP will progress to using other illegal drugs.

The most up to date statistics of stimulant drug use in New Zealand come from the 2005 illicit drug monitoring system (IDMS). From April to August 2005, 78 frequent AMP/METH users were interviewed from five principal New Zealand cities. These participants had to be over 16 years of

age and, in the past six months, to have used AMP/METH monthly. For the purposes of the present study the data that are presented relates to the participants' use of herbal highs. Of all participants, 71% had tried BZP-based substances, and 58% had used them in the previous 6 months. In the previous 6 months, 26% of the participants had binged on them. A drug binge is when the individual takes the drug consistently over periods of up to five days without sleep (Wilkins, et al, 2005) followed by a period of abstinence (Nordahl, Salo, & Leamon, 2003). Interestingly, 24% believed that BZP-based products posed no health risk at all. The researchers concluded that the use of herbal highs was surprisingly high and recommended future research to address the relationship between legal and illegal substances, as individuals using them may not notice a difference in the effects of the drugs (Wilkins, et al., 2005). Additionally, the two drugs that were considered very easy to obtain were cannabis (70%) and METH (52%). The reality that 52 per cent of the participants stated that METH was easy to obtain is surprising and alarming. Since the ingestion of BZP in adolescence may lead to further experimentation with other stimulant drugs, then the apparent easy availability of METH may produce a cohort of adults who will progress to METH. These adults may prove in the future to have a range of problems, arising both from adolescent exposure to BZP and abuse of other stimulants.

A risk factor for later illicit drug use is the easy availability of drugs in an individual's environment (Gilvarry, 2000). This is suggested to have contributed to the increase in experimentation with substances by young people (Ellickson & Morton, 1999). Animal self-administration studies support this phenomenon. For example, rats given unlimited access to self-administer cocaine develop symptoms related to human characteristics of addiction (Ferrario, et al., 2005). Therefore, drug availability can be considered a risk factor for the development of SUD.

Benzylpiperazine use may be becoming part of the youth culture because it is currently readily available throughout New Zealand. Although retailers are not supposed to sell the products to

anybody under 18, like alcohol, they are being consumed by the younger population as a socially acceptable part of an individual's weekend behaviour (The Expert Advisory Committee on Drugs (EACD), 2004). BZP-based drugs have been portrayed as an increasingly common and even essential accessory for a night out (Janes, 2004). The extremely easy availability of drugs can be considered to be compounding the psychological, environmental and genetic risk factors associated with SUD. It is becoming increasingly apparent that much of the substance abuse occurs in adolescent populations, between the ages of 14 and 18 years of age (Riddle, Kokoshka, Wilkins, Hanson, & Fleckenstein, 2002).

Substance use appears to peak during late adolescence and early adulthood and then subsequently declines (Merline, et al., 2004). However, despite this knowledge, there has been only limited research into the possible long-term effects of drug abuse in this age bracket (Andersen, 2005; Laviola, et al., 1999; Smith, 2003). Evidence exists that adolescents show greater addictive potential to drugs of abuse (DOA: Stansfield & Kirstein, 2005). This is particularly concerning when drug usage in this age bracket is increasing, rather than decreasing (Berk, 2001; U.S. Department of Justice Drug Enforcement Administration, 2002; Substance Abuse and Mental Health Services Administration (NSDUH), 2004). New Zealand data on drug use among adolescent populations suggest similar patterns to those seen worldwide. That is, adolescent substance use is increasing rather than decreasing (Ministry of Health, 2001). However, it must be remembered that BZP was introduced into New Zealand after these data had been collected and as yet, BZP-based drugs have not been included in the statistics. Overall, the escalating use of mind-altering substances is important because of the detrimental effects on the health, and social, economic and personal wellbeing of individuals who use them.

In conclusion, substance use and hence SUDs are increasing worldwide. Research with animal models supports the DSM-IV criteria for substance dependence and substance abuse. However, in

human populations there are complex interactions between many psychological, environmental and pharmacological factors. The singling out of a leading risk factor contributing to substance abuse is practically impossible. Yet, to make the transition to substance abuse, an individual must first experiment with substances. It has been proposed that DOA cause either changes in the brain because of continued drug use or that some individuals are predisposed to the addictive properties of drugs. The stage theory of addiction gives some insight into possible consequences of legal BZP use, since it may act as a gateway drug for further use of illegal drugs. This is disturbing as a recent study in New Zealand has suggested that METH is relatively easy to obtain. Additionally, the easy availability of drugs is considered to be a compounding risk factor for SUD. It is predominantly a youth population that is embracing BZP-based drugs and the increasing prevalence of drug use among this cohort of the population is disturbing. Therefore, the implications of BZP use among adolescents may be significant and set the stage for a host of problems in adulthood.

1.3 Adolescence

Adolescence is a transitional phase in development, spanning the years from puberty to early adulthood (Smith, 2003). There is an increase in abstract, independent thought and sexual interest (Gilvarry, 2000). In this crucial developmental period of life, individuals make choices that may strongly influence their adulthood identities and these choices can impact on adult functioning and may be problematic for future adjustment (Rao, Daley, & Hammen, 2000; Stansfield & Kirstein, 2005). Taking drugs during adolescence increases the risk of drug abuse in adulthood (Stansfield & Kirstein, 2005) and evidence exists that adolescents and young adults exhibit higher rates of experimental use of substances than older adults (Chambers, Taylor, & Potenza, 2003). In addition, the adolescent developmental phase is when experimentation with addictive drugs usually begins and longer-term abuse patterns are established (Laviola, et al., 1999; Rezvani & Levin, 2004). This section addresses the risk factors for subsequent drug abuse in adulthood. There is a combination of psychological, environmental and biological risk factors that may interact to make adolescents shift

from experimentation to abuse. Sensation-seeking, peer affiliations, comorbidity and early exposure to drugs are likely risk factors for later drug use.

Sensation-seeking and risk-taking are common adolescent traits. These have been identified as risk factors for substance use (Barnea, et al., 1992; Stansfield & Kirstein, 2005). Sensation-seeking is defined as a need for varied, complex or novel sensations (Dellu, Mayo, Piazza, LeMoal, & Simon, 1993). However, for some individuals, the initial stage of experimentation can escalate into increasing patterns of frequency and risk (Hallfors, Waller, Bauer, Ford, & Halpern, 2005). These adolescent individuals are believed to score highly on the sensation-seeking personality trait (Barnea, et al., 1992). Therefore, increased sensation-seeking may be a developmental trait that strengthens the transition from common adolescent experimentation to SUDs in adulthood. Additionally, during this phase, adolescents spend less time with family members and more time with their peers.

During adolescence the relationship with the peers increases as the relationship with the parents decreases (Berk, 2001). Amongst the strongest predictors of adolescent drug use are the peer affiliations and conformity to the peer groups' values (Spooner, 1999). Pro-drug attitudes and peers may model positive behaviours surrounding the use of drugs and additionally provide opportunities for use (Ellickson & Morton, 1999). Adolescents typically gather in groups of five to seven (Berk, 2001) and if individuals within this group consume drugs like BZP, it may be considered a prosocial activity (Catalano, et al., 1996; Chen & Kandel, 1995). This is immensely important as conformity to peer pressure is prevalent during adolescence (Berk, 2001). Teenagers are curious about adult behaviours, therefore may be curious about drug use. If the individual's peers are consuming legal highs, then it appears safe to assume that with the legal status, peer conformity and pressure, that an individual has ample opportunities to consume BZP. However, not all adolescents are normal adolescents. Some may have a comorbid undiagnosed disorder for which there may be additional

detrimental consequences.

Comorbidity is the co-occurrence of two or more disorders in a single individual (Barlow & Durand, 2002). One common psychological problem encountered in adolescence is depression and it is estimated approximately 15 to 20 per cent of teenagers have had one or more depressive episodes (Berk, 2001). Armstrong and Costello (2002) reviewed all published literature on the comorbidity of substance use and other psychiatric disorders and found that approximately 60% of youths who developed a SUD, had a comorbid diagnosis. They concluded that this highlights the fact that some are not just normal adolescents experimenting with substances but are, in many cases youths with other underlying problems (Armstrong & Costello, 2002; Wills, McNamara, Vaccaro, & Hirk, 1996). The present study used an animal model comparing normal adolescents. Therefore, the concept of comorbidity could not be addressed. However, it is important to mention as there may be worse outcomes for individuals with more than one disorder (Kandel, Huang, & Davies, 2001; Merikangas, et al., 1998). Although, underlying depression or anxiety may increase the risk for substance use, for normal individuals it appears that the earlier the contact with the substance the worse the outcomes.

It has been suggested that the first encounter with addictive drugs is critical, and the earlier this encounter the more likely that dependence on the drug will occur (Adriani & Laviola, 2003; Chambers, et al., 2003; Spooner, 1999). Evidence exists that individuals who have not begun drug use by the age of 20 are unlikely to begin it after this age (Gilvary, 2000; Merline et al., 2004). Illicit drug use can start as early as the age of 12 years, but the peak periods of initiation are between the ages of 15 and 19 years of age (Stansfield & Kirstein, 2005). It has already been established that young people experiment with alcohol before they are legally permitted to (Catalano, et al., 1996). Alcohol and marijuana use by individuals under 18 years of age is an offence. Therefore, as the first encounter with a mind-altering substance is critical, BZP may lay the foundation for continuing

experimentation with other substances. Rather than discouraging early use of substances, this may contribute to the escalating prevalence of substance use among young people.

In summary, adolescence is a crucial transitional phase in development in which there is an increased risk for subsequent drug use in adulthood. The risk factors identified above can predispose an individual to experiment with substances. Normal adolescent behaviour is believed to involve experimentation with DOA. However, there are differences between individuals and within the same individual at different developmental stages of their lives. Human psychological functioning is shaped by both biological (i.e. genetics, brain function, and nutrition) and experience-based factors (i.e. family, school, work and friends), and these factors interact in complex ways (Carlson, 2001). There are therefore, many different risk factors and reasons to why adolescents experiment with DOA, yet the long-term negative outcomes may be the same: a SUD. The changes in the brain during development are proposed to play a fundamental part in the development of a SUD.

1.4 Neurodevelopment

Neurodevelopment and neurotransmission play a critical role in the mechanisms of action of addictive drugs. There is evidence indicating differences in development between adolescence and adulthood in brain regions thought to be important in drug abuse (Spear, 2000). After the synaptic pruning and myelination that typically occurs during adolescence and young adulthood, brain development reaches completion (Andersen, 2005; Rezvani & Levin, 2004; Spear, 2000). Therefore, exposure to drugs during adolescence can have long-lasting implications for brain structure and function (Stansfield & Kirstein, 2005). The adult brain can generally compensate for drug-induced changes within the synaptic clefts, whereas more permanent changes can occur in the immature developing neural systems (Laviola, et al., 1999; Stanwood & Levit, 2004). The transitions from recreational experimentation to the compulsive patterns of drug use and abuse are

understood to arise from change in the neurotransmitter systems (Robinson & Kolb, 2004). Although dopamine (DA) is the principal neurotransmitter involved in DOA, it is proposed in this thesis that serotonin (5-HT) may also be important in BZP effects. Therefore, this section provides a broad overview of the neuroadaptations caused by DA and 5-HT and their relationship with the prefrontal cortex (PFC). The PFC is the last area of the brain to mature fully and any modification of DA or 5-HT activity is likely to cause long-term behavioural changes typically expressed in adulthood. In addition, the theory of neuronal imprinting is considered, as administration of a drug while the brain is still developing may cause long-term behavioural deficits which are secondary to the acute drug effects.

Dopamine is a neurotransmitter involved in memory formation, retention and extinction, movement, reward and predispositions for drug abuse (Millan, 2003; Volkow, et al., 2002). The rewarding effects of drugs that are responsible for continuing use are mediated by the mesolimbic DA system (Piazza & LeMoat, 1998), especially DA projections to the nucleus accumbens (NAc) (Robinson & Berridge, 2001). The NAc is implicated not only in levels of self administration with stimulant drugs (White & Kalivas, 1998) but also in the rewarding properties of all addictive drugs (Andersen, 2005). In the human brain during adolescence, DA is overproduced and then subsequently reduced as the brain develops to its mature state (Bolanos, Glatt, & Jackson, 1998). Additionally, DA may play a critical role in shaping neural responses to drugs during this period and these responses might be quite different in adolescence from those of the same receptors in adulthood (Penner, McFadyen, Pinaud, Carrey, Robertson, & Brown, 2002). However, there is evidence that the neurons containing DA and 5-HT do not necessarily die after drug use. Instead, it is more likely that their nerve terminals are damaged and further development is limited (Fukami, Hashimoto, Koike, Okamura, Shimizu, & Iyo, 2004). Although there is conclusive evidence of involvement of DA and the reward pathways in drug abuse, the same does not characterise 5-HT.

The 5-HT system is involved in the regulation of emotion. Emotion consists of stress, sexual behaviour, memory, cognition, mood, arousal, pituitary hormone secretion and satiety (Carlson, 2001). There is to date functional evidence for the existence of 16 different subtypes of 5-HT receptors (for a review see Naughton, Mulrooney, & Lenard, 2000). Regardless of the different functions of the known subtypes of 5-HT, the 5-HT system is the critical regulator of emotional functioning (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004), principally anxiety, impulsivity and aggression. Additionally, 5-HT integrates complex brain functions such as motor activity, sensory processing and cognition (Lesch & Merschdorf, 2000). Like the DA system, during adolescence the 5-HT system undergoes substantial reorganization. For example, it has been reported that in a rat's hippocampus, the 5-HT levels are five times higher at puberty than at either the juvenile or adult ages (Chen, Turiak, Galler, & Volicer, 1997). The decrease of 5-HT uptake after puberty is suggested to be important for the modulation and attenuation of the rewarding properties of stimulant-like drugs. (Hashimoto, Harumi, & Guromaru, 1992); therefore, the administration of BZP while neurons operated by DA and 5-HT are not fully matured, may have long-lasting implications. As 5-HT is known to be involved in regulation of mood and the expression of emotion, it is, therefore, connected to the development and continuation of many mental illnesses such as anxiety, depression, obsessive-compulsive disorder and panic disorder (Naughton, et al., 2000). As the brain, primarily the PFC, is not fully developed in adolescence and the 5-HT system is still undergoing changes, BZP administered during this period may affect the regulation of emotion.

The PFC is the last area of the human brain to mature (Smith, 2003; Spear, 2000) and 5-HT is known to be involved in its functions. The PFC is implicated in impulsive control, attention and executive function (Andersen, 2005). Executive functioning includes organization, planning, strategising, decision-making and inhibitory control of behaviour (Ferrario, et al., 2005). In other words, the PFC is the brain's behavioural control system (Carlson, 2001). There is conclusive evidence that there are differences in executive functioning between adolescents and adults (Spear,

2000); for example, the underdeveloped PFC in adolescence explains to some extent the use of addictive drugs (Smith, 2003). Research has also shown that rats with PFC lesions are at a greater risk for the development of SUDs (Chambers, et al., 2003) because of loss of control over behaviour, or increased impulsivity (Ferrario, et al., 2005). Therefore, any alteration to the PFC when it is not fully matured could have long-term negative implications for the normal development of behaviour (Stansfield & Kirstein, 2005).

In summary, it is suggested that alterations to the DA, 5-HT systems and the immature PFC could cause behaviour changes which are expressed in adulthood. Although it is not known where and exactly how BZP exerts its stimulant properties on the brain, both DA (Baumann, et al., 2005) and 5-HT (Baumann, et al., 2005; Tekes, Tothfalusi, Malomvolgyi, Herman, & Magyar, 1987) have been implicated in the drug's effects. Nevertheless, effects on these neurotransmitters in rats' brains have not yet been fully researched (see Bauman, et al., 2004; 2005 for a description of this) and there are no published studies of BZP's long-term behavioural effects. The present study therefore aims to throw some light on the latter deficit. If indeed there were behavioural changes observed in adulthood because of adolescent BZP exposure, it would support the neuronal imprinting theory.

1.4.1 Neuronal imprinting

Neuronal imprinting occurs when the effects of the exposure to a drug outlast the drug itself (Andersen & Navalta, 2004). Increasingly, evidence is suggesting that long-term effects of drug exposure in adolescence are delayed and expressed typically during adulthood (Aarons, et al., 1999). McFadyen, Brown and Carrey (2002) state that neural development may continue until approximately 20 – 25 years of age. Therefore, throughout adolescence, the plasticity of the brain continues and also hormonal levels change drastically (Laviola et al, 1999; Smith, 2003). Aarons et al. (1999) have researched alcohol and drug use in human adolescents and suggest that even limited or infrequent use may be associated with adulthood depression, lack of a sense of purpose in life and

lower self esteem

Studies of both adolescent and adult animals suggest that the adolescent brain is more sensitive to the lasting effects of substances than adult animal brains (Smith, 2003). The most conclusive support for this theory comes from studies with methylphenidate (MPH; commonly known as Ritalin). MPH is a stimulant drug used to treat attention deficit hyperactivity disorder (ADHD). Studies administering MPH to adolescent animals have shown behavioural changes long after the drug was withdrawn. For example, after preadolescent MPH exposure, adult animals showed depression in a forced swim task (Carlezon, Mague, & Andersen, 2003). After adolescent exposure to MPH, rats had reduced responsiveness to response to natural rewards (Balanos, Barrot, Berton, Wallace-Black, & Nestler, 2003), increased vulnerability to substance abuse (Brandon, Marinelli, Baker, & White, 2001) and increased anxiety-like behaviour (Balanos, et al., 2003). It is therefore conceivable that BZP administration during adolescence might affect the developing brain and consequently later behaviour, consistent with the neuronal imprinting theory.

Changes in behaviour and psychological functioning as a consequence of drug use are believed to be mediated by the strengthening or reorganization of synaptic connections in specific neural pathways (Robinson & Kolb, 2004). An excellent example of the reorganization or strengthening of the synaptic connection is addiction and subsequent relapses. Substance dependent individuals can relapse months or even years after the discontinuation of drug use, providing strong evidence that drug effects can outlast the initial usage (Robinson & Kolb, 2004).

It was hypothesised that research into DA and 5-HT would show that BZP would affect both the DA and 5-HT systems, but that any behaviour changes in adulthood would have arisen mainly from 5-HT deficits. Because BZP shares stimulant properties with certain other drugs, in the next section comparisons are made with other stimulants.

1.5 Long-term Effects of Exposure to Stimulants

The term “stimulant drug” here is broadly defined as it is beyond the scope of this thesis to discuss the various mechanisms of action of the many different stimulant drugs (see Rothman & Baumann, 2003 for a review). In general, so-called stimulants produce a range of effects in humans, such as increased energy, elevated mood, cardiovascular stimulation and a decreased need to sleep (Rothman & Baumann, 2003), regardless, of the stimulants’ different molecular structures. The adverse effects include insomnia, restlessness, and excessive weight loss, grinding of teeth and impaired sexual functioning. Long term use by humans can start a tendency to suspiciousness that may develop into a full paranoid psychosis that some experts believe continues after abstaining from the drug (Nordahl et al., 2003). There are many types of different stimulant drugs, including AMP, METH, cocaine, MPH and methylenedioxymethamphetamine (MDMA). Regardless of different molecular structures, all stimulant drugs affect the neurotransmitter systems and for purposes of easy clarification in this research the above stimulant drugs are all placed under the same wider term.

There is evidence that use of any of the above-mentioned stimulant drugs leads to similar long-term consequences. As shown in Figure 1 (below) stimulant drug use affects the two neurotransmitters involved in substance abuse. Deficits in DA and 5-HT production lead to a range of negative outcomes that can constitute withdrawal symptoms and mood disturbances. To alleviate these negative outcomes, the individual might continue to use the drug, leading to escalating drug use and a circular pattern that may eventually constitute a SUD. Additionally, a number of behavioural, cognitive and psychological consequences can occur. The DA deficits produce predominantly psychomotor deficits, whereas, 5-HT deficits may produce cognitive behavioural dysregulation. In other words, fundamental aspects of pleasurable human behaviour and survival are compromised by continued use of the stimulants (Rothman & Bauman, 2003).

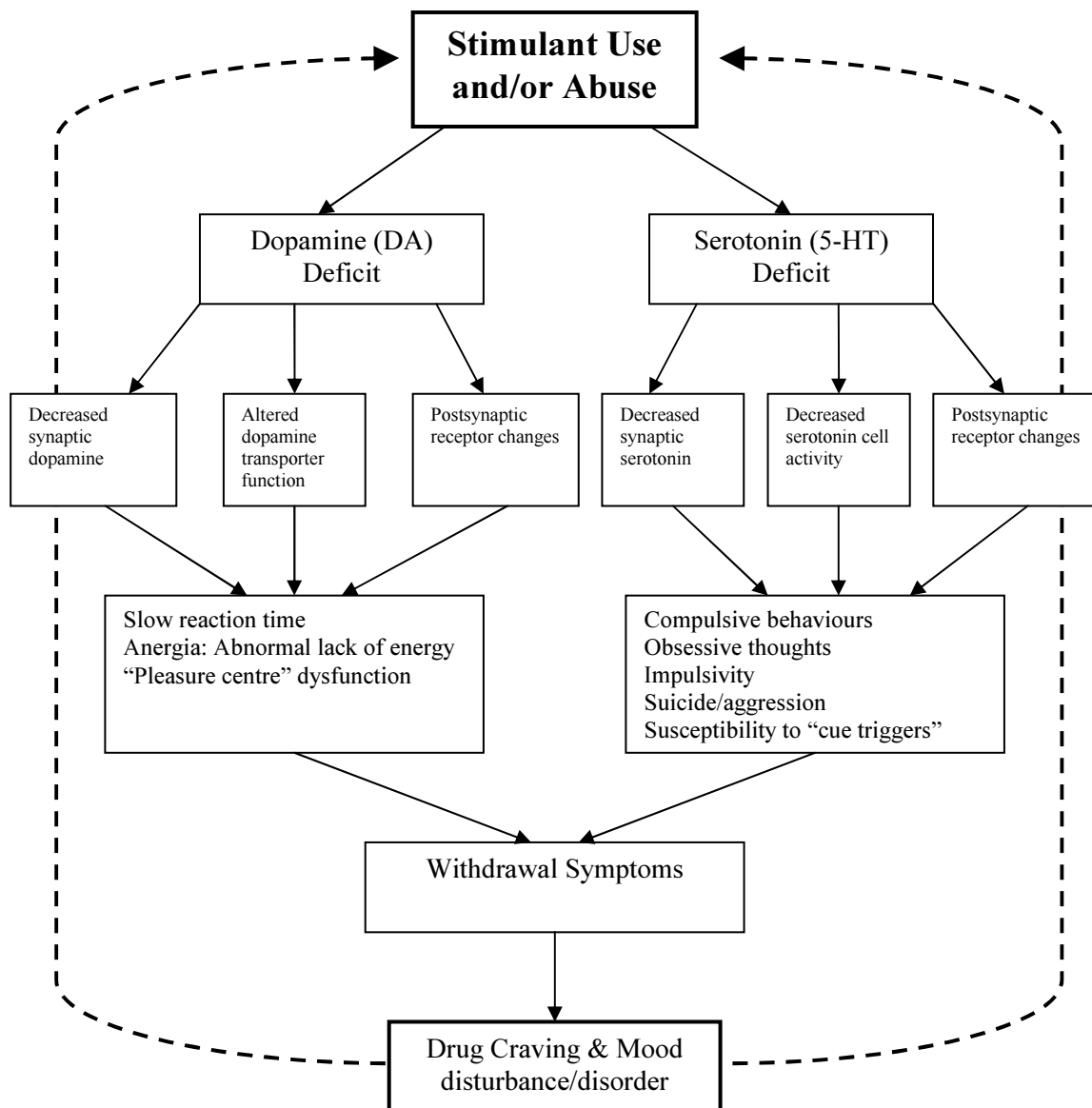


Figure 1. Stimulant use and/or abuse and the corresponding deficits in dopamine and serotonin transmission. Dopamine deficits underlie psychomotor disturbances, whereas, serotonin deficits causes mood disturbances and lack of impulse control, characteristic of withdrawal symptoms, in turn, contributing to increased drug craving and mood disorders. (Adapted from Rothman & Baumann, 2003).

1.5.1 Amphetamine and Methamphetamine

Amphetamine and METH are commonly abused and highly addictive drugs (Rothman & Baumann, 2003). Methamphetamine's chemical structure is similar to AMP, but it has a more potent effect on the central nervous system (CNS; Julien, 2001). That is, METH is the N-methylated analogue of AMP and it metabolizes into AMP in the body. Moreover, it is commonly accepted that METH is more addictive and preferred to AMP by drug users. There are, however, no known neurobiological

differences in action to support this and animals will self-administer both AMP and METH at similar rates (Shoblock, Sullivan, Maisonneuve, & Glick, 2003). Evidence from long-term studies of human METH abuse suggests that detrimental effects occur in tests of memory, mental flexibility and abstract thinking (Simon, Dacey, Glynn, Rawson, & Ling, 2004) and these deficits continue for periods lasting for five days to several years. Additionally, there is evidence that abusers of AMP/METH may develop abnormalities in brain regions implicated in mood disorders. London et al. (2004), found that human abusers of METH provided higher self-ratings of anxiety and depression than controls (London, et al., 2004). In addition, social withdrawal, paranoia and anxiety are well documented occurrences in human METH users (Rawson, Gonzales, & Brethen, 2002). This is supported by animal studies that found marked decreases in social interactions following a brief multiple low dose METH exposure. Clemens et al. (2004), suggest that administration of METH to rats causes decreased social interactions and longer latencies to emerge from a light/dark box when tested 4-7 weeks later, possibly because of a disruption in earlier DA and 5-HT production (Clemens, et al., 2004). If the premise of the present study is correct, then the deficits in neurotransmission that have been found with METH will also occur when BZP is administered. Additionally, as mentioned earlier, because the systems involved in regulating mood are not fully mature in adolescence, there should be an alteration in the human equivalent of mood disorder.

Amphetamine usage in humans is associated with many psychiatric disorders. Rawson et al. (2002), state that depressive symptoms are experienced for a long time after drug discontinuation. This supports the neuronal imprinting theory mentioned above, namely that behavioural and neuroadaptations caused by repeated long-term exposure to stimulant drugs can endure for long periods of time without the drug. Conversely, there is evidence that AMP users are more likely to have an underlying condition of depression that predated the initial drug use. It has been suggested that people suffering from depression will use AMP to alleviate the negative emotional state they are experiencing (Riehman, Iguchi, & Anglin, 2002). In addition to long-term mood disorders, the

altered neuroadaptations may also lead to other psychiatric disorders (Carlezon, et al., 2003) or physical disorders (McCann, Wong, Yokoi, Villemagne, Dannals, & Ricaurte, 1998). The similarities found between the brains of METH abusers and individuals' diagnosed with Parkinson's disease illustrate that METH abuse causes damage to the brain similar to that caused by Parkinson's disease (McCann, et al., 1998). McCann et al. (1998) using positron emission tomography (PET) scans, found that when compared with controls, the abusers of METH had decreased numbers of DA transporters in the putamen and the caudate nucleus, similar to individuals with a diagnosis of Parkinson's disease. This was found even after the subjects had abstained from the drug for approximately three years. In summary, AMP and METH can cause long-term adverse behavioural changes that may lead to escalating drug use in order to reduce depressing or unpleasant experiences caused by continued drug use.

1.5.2 Methylphenidate

Methylphenidate is a stimulant drug used to treat ADHD. Exposure to MPH during adolescence results in different long-term effects from that in childhood (depending on the age of the first exposure). Rats exposed to MPH in adolescence self-administered as adults significantly more cocaine than controls (Brandon, et al., 2001) suggesting that drug exposure in adolescence is a risk factor for future drug use. However, it appears that rats which were administered with MPH in childhood and then tested in adulthood in a place-preference paradigm avoid a cocaine-associated room 33% more often than saline-exposed rats (Carlezon, et al., 2003). These researchers concluded that exposure to MPH in childhood may be a protective agent against future drug abuse. Therefore, the possible beneficial effects of MPH exposure during childhood on future drug abuse, (suggested by Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, (2002) while not conclusive, are of additional interest. However, the findings with adolescent animals further support the view that adolescence is a vulnerable period of development that exposure to drugs at this time may contribute to future drug abuse.

In addition to a possibility of increased drug use in adulthood following MPH exposure during adolescence, there have been other observations. Carlezon et al. (2003) found that preadolescent exposure to MPH can facilitate the development of depression-like behaviours during adulthood. This is consistent with the research done by Bolanos et al. (2003) who found that MPH-treated animals were significantly less responsive to rewards such as sucrose, novelty and sex compared with control animals. They additionally found that the earlier MPH-exposed animals were significantly more sensitive to stressful situations and showed increased anxiety-like behaviours, than the saline-exposed group (Balanos, et al., 2003). They concluded that this effect was because of deficits in the mesolimbic DA system induced by early exposure to stimulants. Further support for long-term deficits in behaviour comes from one of the most researched additions to the stimulant category, namely MDMA.

1.5.3 Methylenedioxymethamphetamine

Methylenedioxymethamphetamine (MDMA, commonly known as ecstasy) is structurally related to other psychomotor stimulants and produces a mixture of stimulant and mild psychedelic effects (Ricaurte & McCann, 2005). MDMA is a serotonin releaser and has been shown in both animal and human studies (Millan, 2003) to increase anxiety in a dose-and test-dependent manner. MDMA produces long-term damage to 5-HT neurons (Green, Sanchez, O'Shea, Saadat, Elliott, & Colado, 2004) and animal studies have shown long-term behavioural and neurotoxic effects of MDMA in rats (Hashimoto, et al., 1992). For example, one study found that, four weeks after exposure to MDMA, animals showed elevated levels of anxiety-like behaviour in the emergence test in the light/dark box. Six weeks after exposure, they showed higher levels of anxiety in the social interaction test, and nine weeks after exposure they showed increased anxiety in the elevated plus maze. The animals' brains were dissected at ten weeks after exposure to MDMA and it was found those previously given MDMA had significantly decreased amounts of 5-HT and 5-hydroxyindoleacetic acid in the striatum, hippocampus and amygdala (Gurtman, Morley, Li, Hunt,

& McGregor, 2002). However, these findings were found after the rats had been exposed to what is considered a high dose regime. To examine whether small doses of MDMA would produce similar effects, rats were administered MDMA in adolescence and tested in adulthood. The MDMA-exposed rats showed reduced social interaction and an enhanced sub-threshold reward effect to cocaine in adulthood (Fone, Beckett, Topham, Swettenham, Ball, & Maddocks, 2002). This suggests that even small amounts of MDMA can cause a long-term change in behaviour. Surprisingly, there is evidence that even a single exposure to MDMA can have long lasting effects on behaviour in rats. These changes are likely to be increased anxiety, and deficits in social behaviour, learning and memory because of the action of MDMA on 5-HT in the PFC (Ho, Pawlak, Guo, & Schwarting, 2004).

McGregor and colleagues (2003) administered MDMA to adult rats and tested them eight weeks later and found increased anxiety in social interaction and light/dark box tests. The authors concluded that MDMA was also associated with poorer memory on an object recognition test. Additionally, pre-treatment with MDMA caused 5-HT loss in the hippocampus, striatum, amygdala and cortex (McGregor, et al., 2003), possibly contributing to increases in the anxiety-like behaviour displayed. Human studies support the findings from animal research. For example, human subjects who have repeatedly taken MDMA have been found to exhibit psychosis, mood disturbances and anxiety disorders (Ricaurte & McCann, 2005). However, a question that remains unanswered is whether individuals who abuse drugs are doing so because of a need to self-medicate or whether the use of drugs leads to the development of other disorders.

1.6 Temporal Ordering

One area of research that is gaining attention is the relationship between psychiatric disorders and substance use or abuse (Brook, Cohen, & Brook, 1998). This is called temporal ordering. There is extensive literature showing a strong association between substance use and mood disorders i.e.,

Blanchard & Blanchard, (1999); Brook, Finch, Whiteman & Brook, (2002); Brook et al., (1998); Chilcoat & Breslau, (1998); Harris & Edlund, (2005); Kandel, et al., (2001); Kandel, et al., (1997); London, et al., (2004); Lopez, Turner, & Saavedra., (2005); McGregor, et al., (2003); Merikangas, et al., (1998); Merline, et al., (2004); Milich, et al., (2000); Morley, Gallate, Hunt, Mallet, & McGregor., (2001); Petraitis, et al., (1995); Rao, et al., (2000); Riehm, et al., (2002); Spooner, (1999); Tomlinson, Tate, Anderson, McCarthy, & Brown, (2005). However, the crucial question is, does substance use precede psychiatric disorders or vice versa (Lopez, et al., 2005)? Most clinical studies have found that psychiatric disorders proceed of substance use disorders (Armstrong & Costello, 2002; Johnson & Kaplan, 1990) and importantly this may affect the transition from experimentation to later abuse or dependence. However, it takes a longer time to reach the DSM-IV diagnostic criteria for a substance use disorder than for a mood disorder; therefore, reports that the mood disorder precedes the substance disorder need to be treated with caution.

Most evidence supports the assumption that anxiety disorders generally precede the onset of substance dependence (Lopez, et al., 2005). There are three possible views concerning the association between substance use and anxiety disorders. One view is based on a causal link from anxiety disorders to substance use in order to alleviate the anxiety symptoms. This is described as the “self-medication hypothesis” (Harris & Edlund, 2005). For example, an individual will use stimulants to decrease inhibition and increase confidence, or conversely will use depressants to decrease tension or anger. An opposing view is that the causality runs from substance use to anxiety disorders (Chilcoat & Breslau, 1998), specifically that substance use may advance the onset of anxiety symptoms. The final view is that there is a shared etiology or a third variable, such as a genetic predisposition (Merikangas, et al., 1998). Merikangas et al. (1998), suggest that genetic factors, environmental risk factors and/or prenatal environment may predispose an individual to both substance use disorders and anxiety disorders. The present study was influenced by the view that causality progresses from substance use to anxiety disorders. However, neither of the other two

approaches should necessarily be discounted because the animals used in the present study were normal rats, without genetically increased anxiety or any differences in environmental or prenatal manipulations. Therefore, it would be likely that, if BZP induces behavioural changes in adulthood, these would have arisen purely from exposure to BZP, relatively independent of other influences.

Clearly, temporal ordering is a controversial and hotly debated topic. To recapitulate, the self medication hypothesis maintains that drug abuse is attributed to an underlying or comorbid disorder that leads the individual to self-medicate to alleviate the negative symptoms. An alternative view is that causality runs from substance use to a mood disorder or a combination of the two. The present purely behavioural study rests on the assumption that the 5-HT system may be involved, with an expectation of an association between adolescent substance use and anxiety in adulthood (Armstrong & Costello, 2002; Blanchard & Blanchard, 1999; Brook, et al., 2002; Carlezon, et al., 2003; Gurtman, et al., 2002; He, Pawlak, Guo, & Schwartinget., 2005; Kliethermes, 2005; McGregor, et al., 2003; Merikangas, et al., 1998; Morley, et al., 2001; Piper & Meyer, 2004). Therefore, the possibility of increased anxiety in adulthood because of adolescent BZP exposure is extremely important because appropriate levels of anxiety are essential for an individual's ability to stay healthy and resist or combat disease (Garau, Marti, Sala, & Balada, 2000).

1.7 Anxiety

Anxiety might be thought of as fear that does not arise from a specific environmental source. Consequently, anxiety in humans is characterized by unrealistic, unfounded fear and emotion (Carlson, 2001), whereas, anxiety in animals is defined by changes in behaviour. (Handley, 1995). The proportion of people in Western countries who experience serious depression or anxiety is estimated to be about one in five. Moreover, adult clinical and epidemiological literature suggests that 50% to over 80% of substance abusers experience at least one other comorbid diagnosis at some stage in their lives (Armstrong & Costello, 2002). There are different clinical classifications of

different types of anxiety, such as generalized anxiety, phobias, panic and post-traumatic stress disorders (Graeff, Netto, & Zangrossi, 1998). Animal behavioral tests provide similar different classifications of anxiety. The three tests that were used in the present study are tests of generalized anxiety disorder (GAD) or unconditioned anxiety (Ohl, 2003; Millan, 2003). Behaviour measured in the Y-maze, light/dark box and the social interaction tests probably reflect the equivalent of human GAD (Morley, et al., 2001; Ohl, 2003; Samyai, Sibille, Pavlides, Fenster, McEwen, & Toth, 2000). The light/dark box has also been regarded as a test of the equivalent of panic disorder (Graeff, et al., 1998), whereas the social interaction paired test may measure social anxiety (File & Seth, 2003). These ethologically appropriate tests are based on a rodent's innate curiosity to explore novel areas (Samyai, et al., 2000) and do not require learning or any positive or negative reinforcers.

Fear- or anxiety-associated responses that humans typically exhibit are escape, avoidance, non-verbal vocalization and/or hypervigilance. Nonhuman animals also display similar kinds of behaviour so that it is now recognized that both species may share common affective states (Palanza, 2001). This is important, as the assessment of anxiety in animals is based on the assumption that human and animal anxiety is similar (Ohl, 2003). Though different in complexity, parallels may be drawn between the animal and the human brain.

It has been suggested that the reciprocal neural circuits linking the PFC, the amygdala and hypothalamus are directly involved in fear and anxiety (Gonzalez, Rujano, Tucci, Paredes, Alba, & Hernandez, 2000; Voigt, Hortnagl, Rex, Van Hove, Bader, & Fink, 2005). Since the introduction of benzodiazepines, 5-HT has been recognised as playing a crucial part in anxiety-like behaviours (File & Seth, 2003). Most research suggests that 5-HT promotes anxiety and drugs that suppress 5-HT functioning will alleviate anxiety (Handley, 1995). Anxiety and drug abuse are inter-connected, possibly through the functioning of the 5-HT system (Gingrich & Hen, 2001). One premise of the

present study is that 5-HT connections to the immature PFC will cause a change in anxiety observed in adulthood. However, there is evidence to suggest that there are also differences in brain functioning between sexes (Bridges & Starkey, 2004; Crick & Zahn-Waxler, 2003; Dominguez, Cruz-Morales, Carvalho, Xzvier, & Brandao, 2003, Hallfors, et al., 2005; Koenig, et al., 2005; Opland, Winters, & Stinchfield, 1995) and, as anxiety is predominantly diagnosed in females (Baranyi, Bakos, & Haller, 2005; Palanza, 2001), sex differences must be considered.

1.8 Sex Differences

Anxiety disorders appear to be predominantly exhibited by women (Baranyi, et al., 2005; Palanza, 2001). However, hardly any animal models use female animals: the majority of research uses only male animals. Consequently, there are few or no investigations of sex differences (Bridges & Starkey, 2004). A literature review investigating anxiety and 5-HT by Blanchard, et al. (1995), revealed that in 750 published studies (ranging from 1991 to 1994) 90% used only male animals, 9% only females and 1% used both sexes (Blanchard, Griebel, & Blanchard, 1995). However, the generally acknowledged higher frequencies of depression and anxiety are more common in women (Palanza, 2001), and can be attributed to different socialization practices. Females are more strongly discouraged from engaging in risk taking activities than males (Guilamo-Ramos, Litardo, & Jaccard, 2005). Nevertheless, the similarities between the sexes are more prevalent than differences between them (Armstrong & Costello, 2002). Yet, epidemiological studies suggest that the majority of drug abusers are males, but more recent prevalence rates indicate that female drug abuse is increasing (Roth, Cosgrove, & Carroll, 2004).

Males use a wider variety of drugs and initiate drug use earlier than females; however, in terms of clinical severity females obtain higher scores than males on physical symptoms and the emotional consequences of drug use. Additionally, female drug users scored higher on the emotional scales because they stated that they were using the drug as a way to alleviate emotional unease (Opland, et

al., 1995). It is, however, questionable whether this is a true sex (as opposed to gender) difference because males may not readily admit to having any emotional issues or problems as openly as females. Adolescent women are almost equally likely to drink, smoke or take illegal drugs as adolescent men, but are also at a greater health risk (Sarigiani, Ryan, & Petersen, 1999). Therefore, the common assumption that drug use is more common in boys than girls fails to address the current cultural context. Evidence is mounting that there is a growing problem of substance abuse in adolescent females (Litt, 2003). As discussed below, differences between sexes may be due to differences in plasticity during maturation of the brain.

Adolescence is an intense period of development of the brain, characterised by extensive pruning of synapses and receptors, and reorganization of many neurotransmitter systems (Spear, 2000). This usually starts earlier for females than for males (Andersen, 2005). There is also a greater over-expression of DA receptors in the NAc and the striatum in males than in females (Andersen, & Teicher, 2000; McCormick, Robarts, Gleason, & Kelsey, 2004). This over-expression may make the male brain more susceptible to stimulation of the DA pathways implicated in addiction (Teicher, Andersen, & Hostetter, 1995). The most common transmitter involved in sex differences in brain development is DA. Male rats have higher levels of D₁ receptors in the NAc than female rats and female rats have a greater rate of DA release and reuptake (Andersen, & Teicher, 2000) and higher DA transporter (DAT) levels in the striatum than male rats (Spear, 2000). Simply, as there is evidence of sex differences in the brain during the transition from adolescence to adulthood, exposure to BZP may affect the two sexes differently.

1.9 Benzylpiperazine

Evidence to date suggests that, although benzylpiperazine (BZP) is seen by some as an alternative to amphetamine drugs, it produces very similar effects (Baumann, et al., 2005; Bishop, McCord, Gratz, Loeliger, & Witkowski, 2005; Bye, et al., 1973; Campbell, et al., 1973; De Boer, et al., 2001;

Fantegrossi, et al., 2005; Peters, Schaefer, Staack, Kraemer, & Maurer, 2003; Staack & Maurer, 2005; Wikstrom, et al., 2004). BZP is the base compound in most herbal highs and amounts of the drug in each tablet can vary from 60 mg to 150 mg (for a review see <http://www.herbalhighs.co.nz/>). However, BZP is not classified as a drug in New Zealand. It is sold at liquor outlets, service stations and dairies without advice, to any age group, with no safety measures or quality control (EACD, 2004). It is marketed as a “herbal high”, that is safe and as an alternative to illegal drugs (Bowden, 2004). Because of the misconception that party pills are “natural” or “herbal”, people appear to take many times the recommended dose, aiming for greater effects (Allan, 2005). Dr Tonia Nicholson from Waikato Hospital surveyed 125 random users of party pills admitted to hospital. One third of the users were between the ages of 14 and 25, one third had not read the instructions and one third had taken more pills than the recommended dose (Jamieson, 2005). This highlights the poor understanding and misuse of party pills by many New Zealand individuals.

Many people in New Zealand are increasingly taking herbal highs, although little is known about their possible long-term effects. This is accompanied by the steady development of an industry: an estimated 1.5 million doses were manufactured in New Zealand in 2003 (EACD, 2004), and an estimated eight million BZP-based doses have been consumed since 1999 (Allan, 2005). No studies have addressed the possible long-term effects of BZP use. Because increasing numbers of individuals are under the impression that BZP is a safe alternative to illegal drugs, more research is needed to clarify its effects. A dramatic example of its adverse effects is that of a 20-year old man who developed a brief psychotic episode 12 hours after the ingestion of ‘Rapture’ (a “herbal high”, produced by Stargate International). The man had no prior psychiatric history, yet displayed delusional beliefs and auditory and visual hallucinations. The authors concluded that some individuals could be more vulnerable to adverse effects of BZP than others (Austin & Monasterio, 2004). However, Bowden (2004) states that his company, Stargate International, started producing the products in 1999, using BZP, amino acids and vitamins as an alternative to illicit and poor

quality AMP/METH then available in New Zealand.

Benzylpiperazine was originally synthesised for use as an anti-parasitic agent to treat infestations of large roundworms and pinworms. It is given orally to cattle, pigs, dogs, cats and chickens and produces paralysis of the parasites, thereby dislodging them and enabling their excretion from the digestive tract (National Chemicals Inspectorate, 2004). However it was subsequently found to reverse the effects of tetrabenazine in rats and mice; therefore it was suggested that BZP may have antidepressant properties (Tekes, et al., 1987). However, it was never marketed as an antidepressant because studies in rodents showed it to have similar effects to AMP: mice administered with BZP showed involuntary head movements, hyperactivity and reduced reaction time in shock avoidance studies, similar to AMP (Miller, Green & Young., 1971, cited in Wikstrom, et al., 2004). Therefore, any therapeutic effects of BZP were dismissed by researchers, as it was concluded that it may have the potential to be abused (Bishop, et al., 2005).

Benzylpiperazine has also been trialled in human population, comparing its effects with that of AMP. In a double blind study by Campbell and colleagues (1973), it was reported that former AMP addicts could not distinguish between the effects of 100mg of BZP and 10mg of AMP, and both were liked by the participants. The subjects reported that the subjective effects of BZP were more enjoyable than AMP (Campbell, et al., 1973). The authors concluded that BZP is a compound with a potential for abuse and recommended that it be placed under statutory control similar to that regulating the use of AMP. A similar double-blind study was conducted by Bye and colleagues (1973), in which 12 healthy volunteers were orally administered either AMP (1 mg to 7.5 mg) or BZP (20 to 100 mg). They were scored on performance tests (that is, tapping, addition and hand steadiness tests, and auditory vigilance tests), cardiovascular responses and self-reported subjective effects. The results suggested that both BZP and AMP similarly produced tachycardia and increases in systolic blood pressure. The authors concluded that BZP was an indirectly acting

sympathomimetic amine similar to AMP (Bye, et al., 1973).

Research with rhesus monkeys and rats supports the view that BZP may be addictive (Fantegrossi, et al., 2005) and can cause deficits in both the DA and 5-HT systems (Baumann, et al., 2004). Baumann et al. (2004) were concerned with recent accounts of drug users ingesting combinations of BZP and trifluoromethylphenylpiperazine (TFMPP). BZP and TFMPP were thought to mimic the subjective effects of MDMA. They therefore sought to investigate the effects of BZP and TFMPP on monoamine neurotransmission in the rat brain. They concluded that BZP and TFMPP combinations produced increases in extracellular 5-HT and DA similar to MDMA and are therefore capable of misuse in human subjects. Additionally, adverse behavioural effects (for example, seizures) were observed in five out of the seven rats that received the higher dose (10 mg/kg) combination of BZP and TFMPP. The drug-induced seizures occurred at a dose that was just 3-times greater than the threshold dose for biological activity. Baumann et al. (2004), suggest that BZP and TFMPP are potentially dangerous, even life-threatening, and that there appears to be a very narrow window of safety. For example, a single i.p. dose of 200 mg/kg given to a guinea pig caused death through tetanic convulsive seizures and BZP is also suggested to lower the threshold for seizures in human epileptics (National Chemicals Inspectorate, 2004). The finding that cocaine-dependent rhesus monkeys will self administer BZP at rates as high or higher than they would for cocaine is equally alarming (Fantegrossi, et al., 2005).

In summary, very little is known about BZP and its effects on the brain. There is to date no published study on any long-term outcomes of BZP ingestion. As the limited evidence suggests, BZP is similar to AMP in its effects on DA and 5-HT levels in adult animals. Moreover, there may be many implications for neuronal changes occurring when it is administered to the adolescent brain.

2.0 The Aims and Hypotheses of this Study

Since no previous study has determined whether there are any long-term effects of adolescent BZP exposure on subsequent adulthood behaviour, the main aim of this study was to investigate possible differences in long-term outcomes between saline- and two BZP-treated groups of rats. BZP is a controlled drug in most countries of the world (Baumann, 2004), but not in New Zealand. Therefore, because BZP is openly and legally available in this country the research seeks to throw some light on possible longer-term consequences of exposure to the drug during adolescence.

Anxiety (or emotionality) was principally investigated because, as BZP was initially trialled as an anti-depressant, it then might affect 5-HT levels in the brain. Alterations in 5-HT are implicated in anxiety disorders (Carlson, 2001) and as the adolescent brain is not fully developed, any neurochemical changes produced by exposure to BZP may cause a long-lasting change in the functioning of the individual in adulthood, consistent with the neuronal imprinting theory.

Furthermore, this study examined the effects of different dosing schedules. In other words, would the animals be better or worse off if they are administered BZP over a shorter time frame than if they are administered the same quantity of drug over a longer time frame? Since, both male and female humans take BZP-based drugs, the study aimed to determine if BZP affected the sexes differently.

3.0 Method

3.1 Subjects

The subjects were 30 male and 30 female PVG/C Hooded rats, from the breeding colony at the University of Canterbury, New Zealand. When 30 days old, the pups were weaned and housed in 525 x 330 x 230mm plastic cages, in groups of three or four of the same sex, for the duration of the experiment. They were housed in a controlled environment (rh 22°C ± 2°C and rh 48% ± 10% humidity), and maintained on a 12 hr light/dark cycle (lights on at 8.00 a.m.) with free access to food (commercial rat pellets) and water. All procedures were approved by the Animal Ethics Committee of the University of Canterbury (see Appendix 1).

The subjects were randomly allocated to three experimental groups. Each group had 10 males and 10 females. In the drug procedure phase animals were exposed daily to either saline or BZP from post natal day (PND) 45 to PND 55. All doses were administered at approximately 13.00 hours. The age at dosing was selected to encompass the adolescent stage of development in rats (Spear, 2000; for a comparison of ages and stages of rat versus human development periods, see Figure 1). The test phase was from PND 72 to PND 95, corresponding to the human equivalent stage of early adulthood in rodents (Spear, 2000).

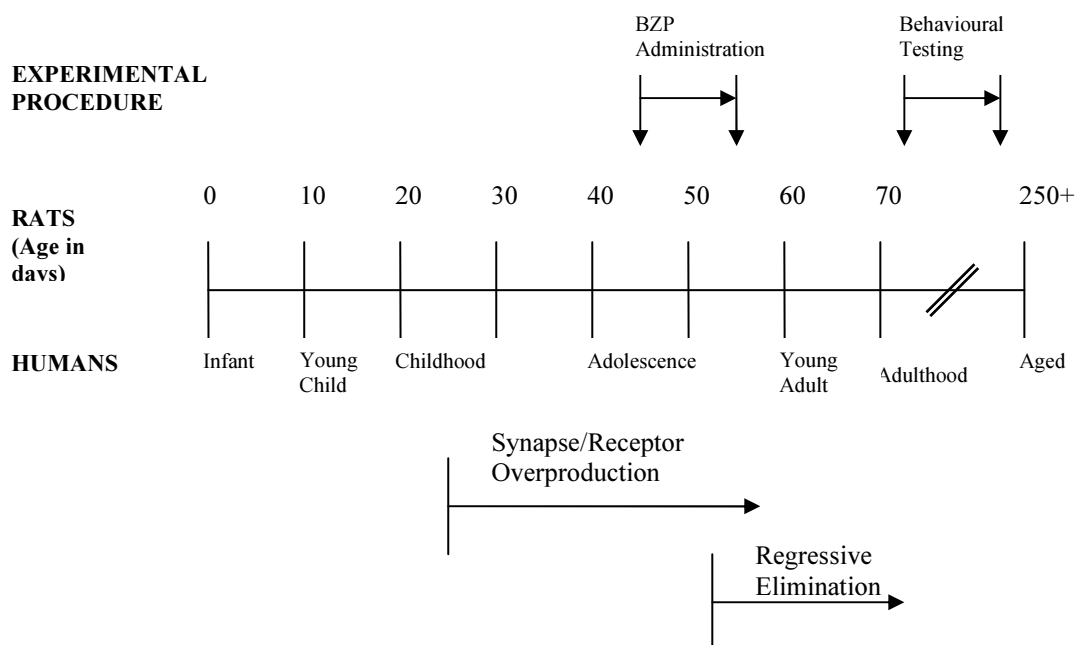


Figure 2. Experimental procedure and a relative comparison of ages and stages of rat versus human development (Adapted from Andersen, 2003).

3.2 Drugs and Rationale for Doses

1-Benzylpiperazine (BZP) was purchased from ABCR GmbH & Co (Karlsruhe, Germany). BZP solutions were mixed daily in sterile 0.9% saline to produce doses of 10 mg/kg and 25 mg/kg, and administered in a volume of 1 ml/kg. From PND 45 to PND 55, (n=20 per group) the animals received a daily intraperitoneal injection (i.p.) of either BZP or saline vehicle. The rats in the “distributed” group received 10mg/kg BZP dissolved in 0.9 % saline daily for ten days. Rats in the “binge” group received 25 mg/kg BZP dissolved in 0.9 % saline for the first four days then saline for the remaining six days. Control rats’ received the same volume of 0.9 % saline (See Table 1 below). Although human users typically take BZP orally, an i.p. route of administration was chosen for ease of drug delivery. All doses were administered at approximately 13.00 hours. Body weights were

monitored throughout drug treatment and all behavioural testing.

Table 1: Days of treatment with BZP (mg/kg) or saline (S) from PND 45 for a total of 30 male and 30 female hooded rats

Group	Days of BZP treatment	1	2	3	4	5	6	7	8	9	10	Total BZP exposure
Control	Male 0 (10)	S	S	S	S	S	S	S	S	S	S	0
Control	Female 0 (10)	S	S	S	S	S	S	S	S	S	S	0
Distributed	Male 10 (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	100 (mg/kg)
Distributed	Female 10 (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	BZP (10)	100 (mg/kg)
Binge	Male 4 (10)	BZP (25)	BZP (25)	BZP (25)	BZP (25)	S	S	S	S	S	S	100 (mg/kg)
Binge	Female 4 (10)	BZP (25)	BZP (25)	BZP (25)	BZP (25)	S	S	S	S	S	S	100 (mg/kg)

The LD50 in both male and female rats is 2,600 mg/kg (National Chemicals Inspectorate, 2004). Therefore, the BZP doses used in this study can be considered small. The dosing procedures, however, were based on those for the stimulant drug AMP (Vorhees, et al., 2005) and from prior knowledge that BZP's potency is one-tenth that of AMP. A typical 'small' dose of AMP for rats is 1 mg/kg (Robinson & Kolb, 2004). A comparable small dose of BZP would therefore equate to 10 mg/kg. Additionally, one day in a rats' life is equivalent to approximately 10 days in humans (Cho, et al., 2001). Therefore, the distributed group was administered BZP to correspond approximately to weekend use in humans. The higher BZP dose of 25 mg/kg (binge group) was based on research showing contralateral turning behaviour similar to a 'high' AMP dose of 2 mg/kg (Oberlander, et al., 1979) and the total four BZP exposures over four days matched the overall total of 100 mg/kg given to the distributed group. Human binge users of stimulants generally binge on the drug persistently for 2-4 days (Davidson, Lee, & Ellinwood, 2005). Although not precisely consistent with a human binger, this dosing regime would assess both a smaller quantity of BZP taken over a longer period of time, and a larger quantity of BZP taken over a shorter period of time.

3.3 Apparatus and Behavioural measures

This study utilized three empirically supported measures of anxiety-like behaviour (File & Seth, 2003). In the Y-maze a more anxious animal will enter a novel arm less, spend less time in it and avoid the novel arm as their first choice more often than less anxious animals. In the social interaction test a more anxious animal will display less rearing behaviour (outer squares and inner unprotected squares) and spend less time in social interaction compared with normal animals. In the light/dark box more anxious animals will take longer to emerge from the start chamber than less anxious animals. Additionally, total numbers of boli were counted in the light/dark box and social interaction test. A more anxious animal will defecate more than a less anxious animal (Wills, Wesley, Moore, & Sisemore, 1983). Testing in these mazes 2 weeks after drug administration serves two purposes. Firstly it allows the animal to reach adulthood and secondly, it indicates that any behavioural change is not due to drug-induced hyperthermia or any other acute drug effect (He, et al., 2005).

The experimental room was kept at 20°C, with 38% humidity and with dim (47 lux) room lighting. All testing took place between 10.00 and 15.00hrs during the light phase of the rats' light/dark cycle. Each animal was tested in either the Y-Maze or the social interaction test (randomly determined), and then placed in the light/dark box. There was a break of at least two days between each series of tests, to control for habituation effects. Each animal was tested in the Y maze four times, the social interaction test twice and the light/dark box six times. Averages of all of their trials in each test setting were determined.

3.3.1 Responsiveness to Change in the Y-Maze.

The Y-maze taps into rats' innate tendency to explore novel areas (Hughes, 2001; Hughes & Neeson, 2003; Samyai, et al., 2000) and normal animals will enter the novel arm more than the familiar arm, whereas animals with increased anxiety will show less of a preference for novelty.

The enclosed unpainted wooden Y-maze sat on a 700mm-high table and was illuminated by dim (47 lux) fluorescent, overhead (1.480mm above maze) lighting. The maze consisted of two 45-cm long arms and a 30-cm stem. The arms and the stem were 10 cm wide and 14 cm high, and the angle between them was 120°. Each arm of the maze contained a removable black or white insert, which occupied the width, height and 40 cm of the length of the arms. The animals were placed in the first 15 cm of the stem and allowed to freely roam the entire maze for 6 minutes (acquisition trial). The rat was then removed and placed in a covered holding cage (530 x 330 x 230) while the white and black inserts were replaced with two clean black inserts, and the entire maze cleaned with a solution of 20% Powerquat blue and 80% water. It was then replaced in the first 15 cm of the stem and allowed to freely roam the entire maze for 3 minutes (retention trial).

Arm entries were recorded by the experimenter using a PC keyboard, computer and suitably developed event recording software. The dependent variables measured in the retention trials were: (1) the first arm entered i.e. changed (novel) or unchanged in terms of arm brightness; (2) percentage of time spent in the novel arm for the first minute; (3) the percentage of entries made into the novel arm for the first minute; (4) the total time spent in both arms for the first minute; (5) the total entries of both changed arms for the first minute.

All animals were tested in this maze four times. The novel arm was on the left for half the trials and on the right for the other half (randomly determined). The average of the four trials became the representative score for each rat in each measure. Results for the first minute only were recorded because it appears that rats' interest in the novel arm disappears after the first minute (Hughes & Kleindienst, 2004).

3.3.2 The Social Interaction Test

From PND 72 pairs of unfamiliar animals were tested twice in the social interaction test, which is a

test of anxiety and curiosity (Wills, et al., 1983). As with the other tests, it does not require training and the use of shock or deprivation. The animals were weighed on each of the two days preceding the social interaction test and the weights of each pair did not differ by more than 10 g. The apparatus consisted of a wooden open field (OF) 600 x 600 x 250mm, that was placed on a table 700mm high in the same experimental room described above. The apparatus floor was painted flat black and divided into a 4x4 grid of 16 identical squares (twelve peripheral and four central), each measuring 150 x 150mm. The wooden walls were 250mm high and painted flat black. Uncertainty (and thus anxiety) is increased by placing animals in an unfamiliar environment, and by altering the light levels (Garau, et al., 2000). In this case, the light level was low (47 lux) and the test arena was unfamiliar, producing low to moderate anxiety (File & Seth, 2003). An infrared video camera was mounted on a single wooden arm 850 mm above the OF. The camera was connected to a video recorder in a separate room, to record the animals' behaviour for later analysis. The tests were videotaped for 5 min and all testing was carried out between 10.00 and 15.00 hr. The OF was washed (20% Powerquat blue and 80% water) and dried between each pair of animals. The animals were tested with at least a two to three days interval in between each test. Behaviour was scored manually by the experimenter.

Unfamiliar same-sexed rat pairs were matched on the basis of body weights and treatment conditions and placed in the middle of the OF facing each other for the start of the social interaction test. They were allowed to freely roam the entire OF together for five minutes. The animals were assessed for both their individual behaviour and their social interaction behaviour. The individual measures in the OF for each animal for the recorded five minutes were: (1) the total number of lines crossed (locomotion); (2) the total rearing in the outside 12 squares (outer rearing); (3) the total rearing in the inside four squares (inner rearing). The social interactive behaviour for each pair of animals was the total time spent in social interaction (e.g. sniffing, grooming, mounting, and crawling over, crawling under or following the partner). Because the behaviour of one animal affects

that of the other, each pair of rats was treated as a unit (File & Seth, 2003) and only one score per pair was used for measures of social interaction behaviour. Lower levels of social interaction are considered to be indices of higher anxiety (Baranyi, et al., 2005). The number of faecal boli (defecation) deposited by each pair was also recorded as a measure of emotionality (Wills, et al., 1983) -higher numbers of boli reflect higher anxiety. Each animal completed this task twice and the measures were averaged over the two trials.

3.3.3 Light/dark Box Emergence Test

After either testing in the Y-maze or a social interaction test all animals were then observed in a light/dark emergence box. This test is based on rodents' innate aversion to bright illuminated areas. In response to mild stressors, that is, bright light and a novel environment, a rodent faces a natural conflict between the tendency to explore and the initial tendency to avoid the unfamiliar (Bourin & Hascoet, 2003). This apparatus is quick and easy to use and does not require food or water deprivation, shock administration or prior training. It comprised a 200 x 150 x 200-mm high darkened start box, which was painted flat black inside and could be opened (via a sliding wooden door) to a larger 500 x 400 x 200-mm illuminated arena. The apparatus was constructed from wood apart from the floor and ceiling of the arena. The floor consisted of translucent white Perspex that was illuminated from underneath by two 16-lux fluorescent tubes. The light measurements were, 80 lux in the centre of the illuminated area and 172 lux on the floor. The roof of the arena was made of fine wire mesh.

The animals were placed in the smaller darkened box for 60 seconds, and then the sliding door to the illuminated arena was opened. By means of a hand-held stopwatch the experimenter timed how long it took for the animal to fully emerge (all four paws) into the illuminated arena. If the animal did not emerge within five minutes, the trial was terminated and the rat assigned a score of 300 s. All animals were tested this way six times with an interval of at least two to three days in between

each test. The mean of the six trials was calculated for each rat.

4.0 Statistical Analyses

The major focus of this study was to examine longer-term outcomes following adolescent exposure to BZP. The research hypothesis was that animals exposed to BZP in adolescence would demonstrate higher levels of anxiety in adulthood than control animals. The raw data was analysed with ANOVAs using the statistical programme *Statistica 7*. Separate 3 (treatment group) x 2 (sex) ANOVAs were performed on each measure, followed by post hoc Tukey HSD tests when a main effect or interaction was significant ($p < .05$). In the Y-maze, single sample *t*-tests were also computed for first choice, % of novel time and % of novel entries compared with a mean expectancy of 50%. Sex differences were assessed because, although there is a difference between the sexes in activity, little evidence exists for differences in measures of anxiety-like behaviour (File & Seth, 2003).

5.0 Results

5.1 Y-maze Results

As each animal was tested in the Y-maze four times, averages of these four trials were used in the analyses. Separate ANOVA results for all five measures in the Y-maze are depicted in Table 2. Specific post hoc comparisons were made by means of Tukey HSD tests.

Table 2: Mean (\pm S. E. M.) percent of first choice, and entries in novel and both arms and time spent in novel arm, and total time and total entries of both arms for control (n=20), distributed (n=20) and binge (n=20) BZP treated animals, and for male (n=30) and female (n=30) rats, and results of *F* tests.

Treatment group	Control	Distributed	Binge	<i>F</i> (2,54)	<i>P</i>
First Choice	73.75 (4.96)	58.75 (5.21)	46.25 (6.35)	5.99	.004
Total Time (sec)	28.59 (1.95)	17.89 (1.45)	13.84 (1.99)	19.83	.000 ^a
% Novel Time	56.57 (2.86)	46.01 (4.60)	29.05 (5.68)	9.08	.000
Total Entries	2.22 (0.21)	1.51 (0.18)	0.99 (0.11)	14.87	.000
% Novel Entries	58.64 (3.01)	47.39 (4.17)	25.48 (5.10)	15.58	.000

Sex	Male	Female	<i>F</i> (1,54)	<i>P</i>
First Choice	58.33 (4.99)	60.83 (4.89)	0.148	.702
Total Time (sec)	19.36 (2.22)	20.86 (1.42)	0.58	.451 ^a
% Novel Time	41.20 (4.03)	46.56 (4.36)	1.01	.318
Total Entries	1.27 (0.14)	1.87 (0.17)	10.41	.002
% Novel Entries	42.86 (4.09)	44.81 (4.37)	0.16	.694

^a Treatment group X Sex interaction significant ($F(2,54) = 4.73, p < .05$), see text and Figure 5.

As shown in Table 2, for all five measures there was a significant group effect and one sex effect.

Figure 4 illustrates the significant interaction for total time spent in both arms.

5.1.1 First Choice.

As shown in Figure 3, the control rats entered the changed arm first 74% of the time, the distributed group entered it 59% of the time, whereas, the binge animals entered it only 46 % of the time.

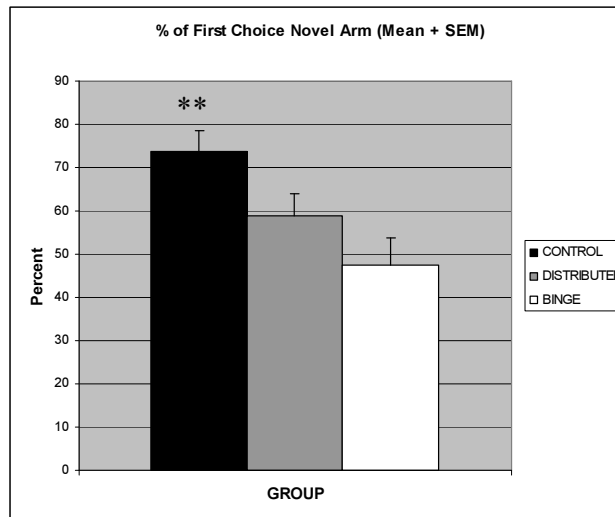


Figure 3: Mean percentage of time the novel arm was entered first for control (n=20), distributed (n=20) and binge (n=20) groups. The error bars represent the standard errors of the means. ** Significantly greater (one-sample *t* test, *df*=19, *P*<.0001) than a chance expectancy of 50%.

The graph indicates that the control animals preferred the novel arm first considerably more often than the other two groups. It also indicates that the distributed group chose the novel arm first significantly more often than the binge group (*p* <.01). Additionally, the control animals preferred the novel over the familiar at a greater than chance expectancy of 50%

5.1.2 Total Time

The significant group effect in total time spent in both arms is more appropriately described in terms of the significant group X sex interaction (see Fig 4)

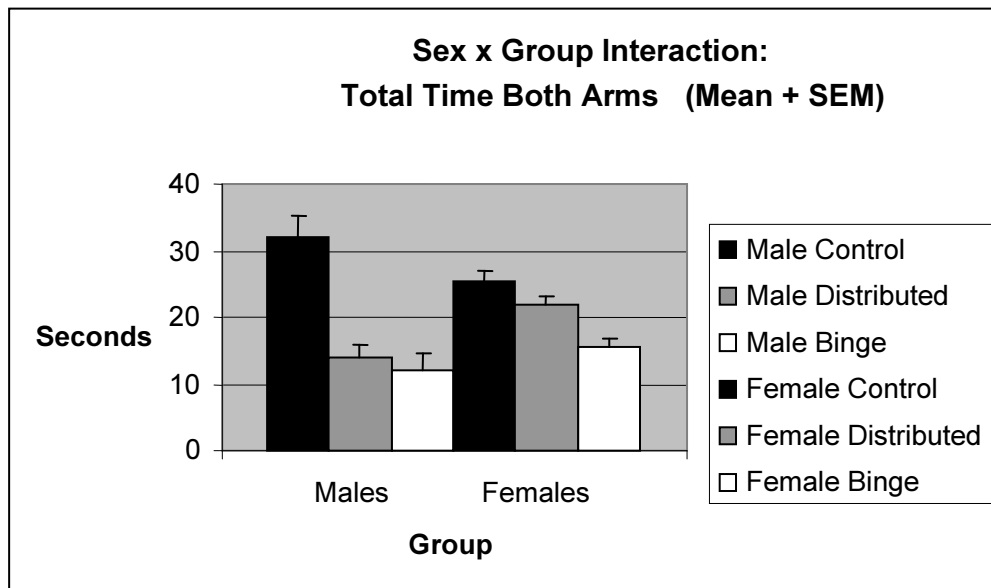


Figure 4: Sex x Group interaction in total time spent in both arms of the Y maze for the control (n=10 Males and 10 Females), distributed (n=10 Males and 10 Females) and binge (n=10 Males and 10 Females) groups. The error bars represent the standard errors of the means.

It is apparent that male rats were more affected by the BZP treatment than females. Post hoc Tukey HSD tests revealed a significant difference between control and distributed ($p < .001$) and control and binge ($p < .001$) groups for males. The same comparisons for females were not significant.

5.1.3 % Novel Time

Results for the percentage of time spent in the novel arm indicate that saline-exposed animals spent significantly more time in the novel arm of the maze than the BZP-treated animals. (See Fig 5).

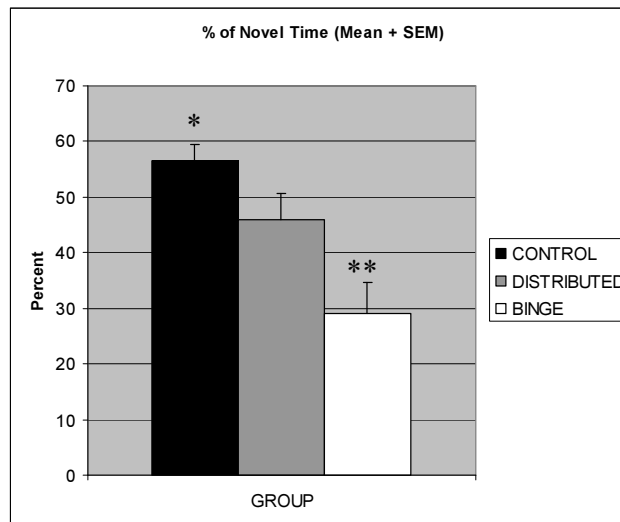


Figure 5: Mean percentage of time spent in novel arm. For the control (n=20), distributed (n=20) and binge (n=20) groups. Error bars show the standard errors of the means. * Significantly greater (one-sample *t* test, *df*=19, $P<.05$) than a chance expectancy of 50%. **Significantly lower (one-sample *t* tests, *df*=19, $P<.001$) than a chance expectancy of 50%.

Control animals spent over half their time (57%) in the novel arm, whereas, the distributed spent just under half (46%) and the binge animals spent less than one third of their time (29%) in the novel arm. The difference was significant between the binge group and both the distributed ($p <.05$.) and the control groups ($p <.01$.). Additionally, the control animals significantly preferred the novel arm, whereas the binge animals preferred the familiar arm.

5.1.4 Total Entries

The total number of entries of both arms outlined in Figure 6, shows that control rats made significantly more entries than treated rats. The difference was significant between the control and both the binge ($p<.001$) and distributed ($p<.05$) groups. As shown in Table 2, female rats made significantly more entries into both arms than males.

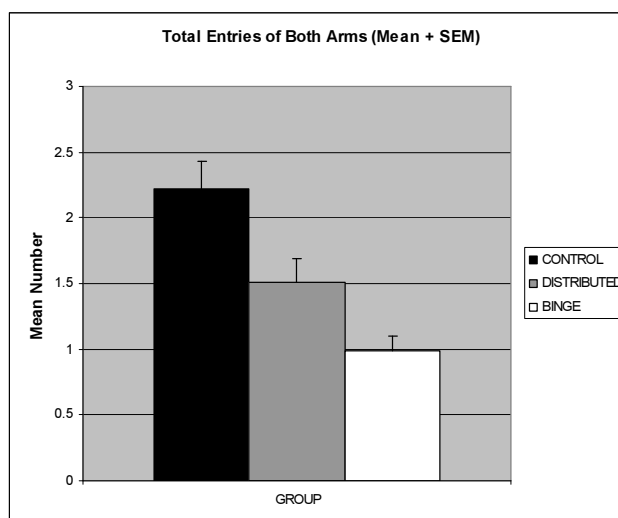


Figure 6: Total entries of both arms for control (n=20), distributed (n=20) and binge (n=20) groups. Error bars show standard errors of the mean.

5.1.5 % Novel Entries

The percentage of entries into the novel arm for each group is shown in Figure 7.

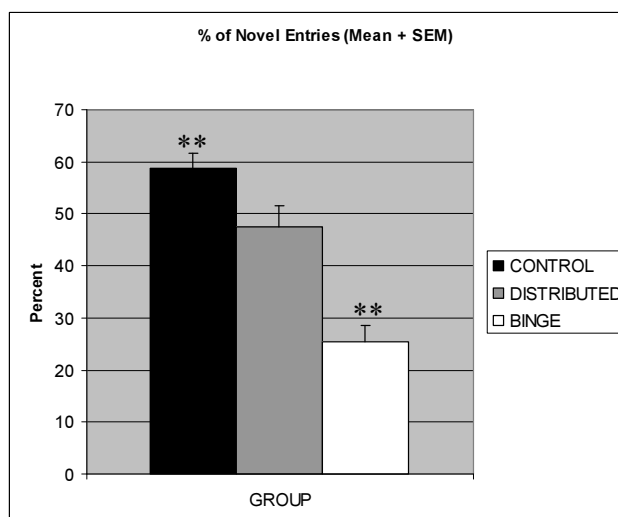


Figure 7: Total percentage of entries in the novel arm for control (n=20), distributed (n=20) and binge (n=20). The error bars show standard errors of the means. ** Significantly greater or lower (one-sample *t* tests, *df*=19, *P*<.01) than a chance expectancy of 50%.

Whereas, the control animals' entries of the novel arm were 59% of the total, comparable rates for the distributed and binge groups were 47% and 25% respectively. The difference was significant

between the binge group and both the distributed ($p < .05$.) and the control ($p < .01$) groups. The control animals made significantly more novel arm entries (compared to a chance expectancy of 50%) than binge animals that made significantly more familiar arm entries.

5.2 Social Interaction Results

As each animal was tested in the social interaction test twice, averages of these two trials were used in the analyses. The ANOVA results are presented in Table 3.

Table 3: Mean (\pm S. E. M.) for locomotion, outside and inside rears, interaction time (secs) and total number of boli for control (n=20), distributed (n=20) and binge (n=20) BZP treated animals, and for male (n=30) and female (n=30) rats, and results of F tests.

Treatment Group	Control	Distributed	Binge	F (2, 54)	P
Locomotion	104.78 (4.61)	108.27 (3.75)	106.80 (3.49)	0.36	.696
Outside Rears	40.00 (1.07)	36.45 (1.20)	36.75 (1.20)	2.84	.067
Inside Rears	6.42 (0.64)	3.57 (0.46)	3.20 (0.37)	12.29	.000
Interaction Time	120.97 (4.09)	103.13 (4.99)	99.13 (3.35)	7.87	.001
Boluses	1.50 (0.51)	1.55 (0.53)	0.85 (0.45)	0.67	.515

Sex	Male (n=30)	Female (n=30)	F (1, 54)	P
Locomotion	94.51 (2.56)	118.71 (2.06)	51.82	.000
Outside Rears	36.71 (0.92)	38.75 (1.01)	2.27	.137
Inside Rears	3.93 (0.47)	4.86 (0.48)	2.58	.114
Interaction Time	112.76 (3.85)	102.73 (3.56)	4.39	.040
Boluses	2.03 (0.45)	0.56 (0.29)	7.12	.010

5.2.1 Locomotion

There was no significant group effect on locomotion, meaning that all groups engaged in approximately the same amount of locomotion. Additionally, there was no sex x group interaction.

However, this measure was significantly higher for females than for males.

5.2.2 Outside Rears

For outside rears there were no significant differences for groups, or sex or a significant interaction effect.

5.2.3 Inside Rears

There was a significant group effect for inside rearing, which was mainly due to a significant difference between the control and both the distributed ($p < .001$) and binge groups ($p < .001$, see Figure 8).

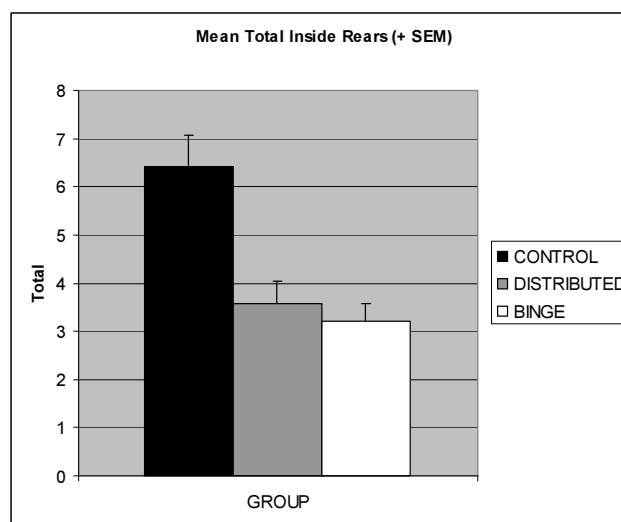


Figure 8: Inside rearing scores for control (n=20), distributed (n=20) and binge (n=20) groups. Error bars denote standard errors of the means.

The control group reared approximately twice as often as the two treated groups. There was no significant difference for sex or a sex x group interaction.

5.2.4 Social Interaction (secs)

There was a significant group effect for social interaction time (see Figure 9) due to a significant difference between the control group and both the distributed ($p < .01$) and binge group ($p < .001$).

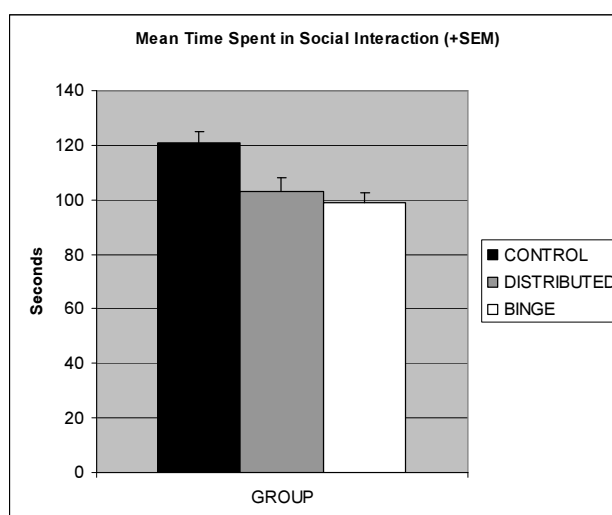


Figure 9: Time spent in social interaction with an unfamiliar rat for the control (n=20), distributed (n=20) and the binge (n=20) groups. The error bars denote standard errors of the means.

As shown in Table 3, male animals spent more time than females in social interaction.

5.2.5 Social Interaction Faecal Boli

Although there was no significant group effect for this measure, male rats defecated significantly more often than females. There was no significant sex X group interaction.

5.3 Light/dark Box Results

As each animal was tested in the emergence box six times, averages of these six trials were used in the analyses. Table 4 shows the ANOVA results for the two measures in this test. i.e., time to emerge, and faecal boli deposited in the dark side of the box.

Table 4: Mean (\pm S. E. M.) for the total time taken to emerge from the dark chamber into the illuminated chamber and total number of boli for control (n=20), distributed (n=20) and binge (n=20) BZP treated animals, and for male (n=30) and female (n=30) rats, and results of *F* tests.

Treatment Group	Control	Distributed	Binge	<i>F</i> (2, 54)	<i>P</i>
Total Emergence Time (sec)	47.57 (10.03)	109.63 (15.99)	90.27(13.13)	5.77	.005
Total boluses	1.80 (0.47)	0.65 (0.24)	0.70(0.28)	4.13	.021
Sex	Male (n=30)	Female (n=30)		<i>F</i> (1, 54)	<i>P</i>
Total Emergence Time (sec)	95.06 (12.35)	69.92 (10.66)		2.71	.015
Total boli	1.63 (0.33)	0.46 (0.20)		9.98	.002

5.3.1 Emergence time

There was a significant group effect for the time it took to emerge from the darkened box into the brightly alit arena (see Figure 10).

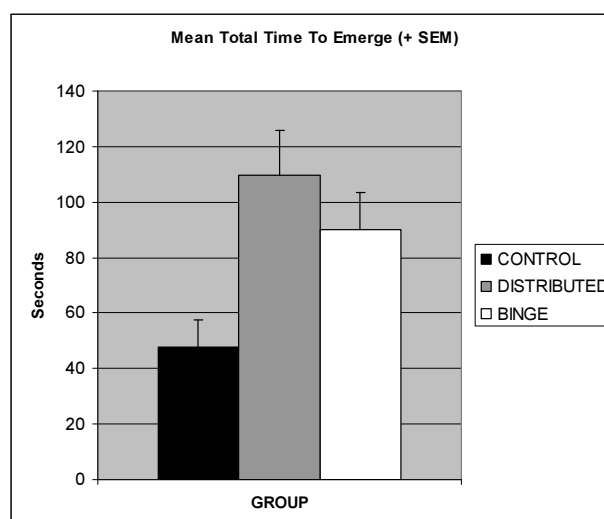


Figure 10: Time in seconds for control (n=20), distributed (n=20) and binge (n=20) to place all four paws into the illuminated arena. Error bars show standard error of the means.

The distributed group took significantly longer to emerge from the darkened space than the control group ($p < .01$), and males took longer to emerge than females ($p < .015$).

5.3.2 Light/dark Box Faecal Boli

The group effect was significant (see Figure 11) for faecal boli which was due to a difference between the control and both the distributed ($p < .05$) and the binge group ($p < .05$). As outlined in Table 4, male rats defecated significantly more often than the females in the darkened area of the light/dark box.

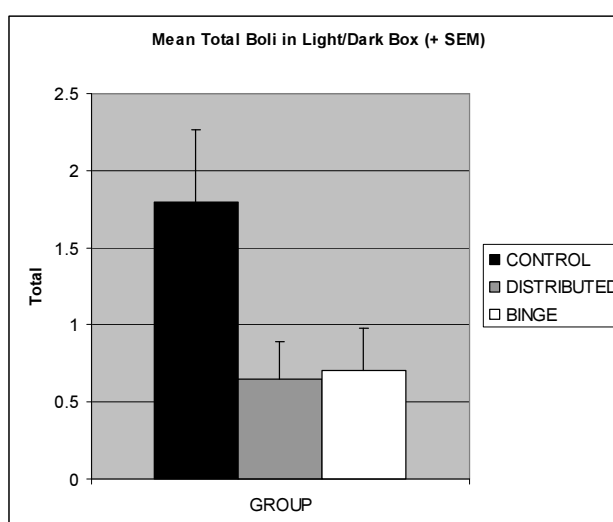


Figure 11: Mean total of boluses over six tests in the emergence test for control (n=20), distributed (n=20) and binge (n=20). Error bars show the standard error of the means.

6.0 Discussion of Results

The present study randomly exposed adolescent rats to either a saline or one of two different BZP-administered groups. All groups received either the vehicle or the drug during their adolescent stage of development (PND 45 – PND 55; Spear, 2000). The control animals received daily i.p injections of saline, the distributed group received daily 10 mg/kg BZP, whereas, the binge group received four days of BZP 25 mg/kg and then saline for the remaining six days. The rats were subsequently tested in three empirically validated measures of emotionality in adulthood.

6.1 Summary of Results

The results showed that there were systematic differences in measures of emotionality in the predicted direction, in almost all of the behavioural tests used. That is, as adults, the rats that had been exposed to BZP as adolescents displayed more emotionality than control rats. This can be summarized as follows. In the Y-maze the treated animals showed no preference for the novel arm as their first choice, spent less time in it, made fewer entries into both arms and fewer entries into the novel arm, than control animals. In the social interaction test, the BZP-exposed animals reared less in the unprotected central squares and spent less time in active social interaction, than the control animals. Additionally, in the emergence test, the BZP-treated animals took a significantly longer time to emerge, than control animals. The results of these three behavioural measures support the main hypothesis: adolescent rats exposed to BZP show increased emotionality in adulthood.

Literature on animal models of emotionality state that increased defecation is a sign of increased anxiety (Wills, et al., 1983). Paradoxically, the control animals, (especially males), defecated significantly more often in the light/dark box than the two BZP-treated groups. This would suggest that the control animals were more emotional than the BZP-exposed animals. However, according to veterinarian journals, BZP, when administered to animals as an anti-parasitic, decreases

defecation and increases urination (National Chemicals Inspectorate, 2004). Therefore it is likely that a similar effect was caused by BZP in the present study, thereby accounting for the paradoxical decrease in defecation. However, this clearly needs to be explored further.

The second aim of this research was to see if there was a difference between the two BZP-exposed groups, i.e., would less BZP exposure over a longer time frame result in increased or decreased emotionality when compared with higher BZP exposure over a shorter time frame? It is unknown which dosing regime is more likely to produce detrimental outcomes in adulthood. Long-term behaviour changes have been observed from a single MDMA exposure (Ho, et al., 2004), after brief low MDMA doses (Clemens, et al., 2004; Fone, et al., 2002), and after neurotoxic high MDMA dosing regimes (Gurtman, et al., 2002). Both BZP-treated groups in this study displayed significantly higher levels of anxiety-like behaviours than controls. However, the results suggest that the binge-treated animals displayed significantly higher levels of anxiety than the distributed group. This implies that higher BZP exposure over a shorter time frame is possibly more detrimental for the development of anxiety than a smaller quantity over a longer time frame.

In the Y-maze, animals in the binge condition displayed significantly more anxiety-like behaviour than the distributed group. In summary, the binge rats significantly avoided the novel arm as first choice more than the distributed group. They also spent less of the total percentage time in the novel arm than the distributed group and appeared to have actively avoided the novel arm, preferring to spend time in the familiar arm. This was supported by the lower percentage of novel entries. The binge animals, compared with distributed animals, entered the novel arm less frequently and appeared to prefer entering the familiar arm to entering the novel arm. The differences between the two BZP-exposed groups were not significant however in the light/dark box and the social interaction test measure. Nevertheless, the means were lower for the binge animals compared with the distributed group.

All measures in this study examined anxiety-related avoidance. Therefore, the additional significant difference between the two BZP-exposed groups in the Y-maze suggests that this test may have been measuring something in addition to emotionality. Evidence exists that the Y-maze is also a measure of short-term recognition memory (Hughes, 2001). It has been suggested that rats with functional spatial memory explore the novel arm more than the familiar, while rats with impaired spatial memory explore both arms similarly (Conrad, Lupien, Thanasoulis, & McEwen, 1997). This is a form of memory testing that is dependent on an intact hippocampus. The hippocampus is involved in spatial memory and is also implicated in the regulation of anxiety (Piper & Meyer, 2004). Therefore, the binge-exposed animals may have had a greater impairment in the hippocampus or simply may have displayed more anxiety than the distributed animals. The fact the familiar arm was preferred by the binge-dosed animals shows that they were able to remember the changed arm, so that they could actively avoid it. Regardless, of different possibilities, adolescent exposure to BZP in a larger quantity over a shorter period of time causes greater emotionality in adulthood than the same total quantity of BZP over a longer time frame.

In the light/dark box, there was a difference between the control animals and the treated animals. The septum and hippocampus 5-HT activity constitute the behavioural inhibition system. It is suggested that this system plays an important role in the control of and regulation of anxiety (Dominquez, et al., 2003; McGregor, et al., 2003). However, in this thesis, the distributed group took a longer time to emerge compared with the binge group: this result goes against every other trend observed. This was not statistically significant, yet, it raises a few concerns that warrant further studies. It would appear that, on this measure, the distributed group displayed the highest amount of emotionality. The mean over six trials was used in the analysis and it was observed on the first few trials that the binge animals emerged instantaneously. This appears to have skewed the results, apparently indicating that the binge group was less anxious than the distributed group. A suggestion to explain this finding might be that the binge treated animals were displaying higher impulsivity on

this test. There is evidence that impulsivity is not a one-dimensional construct, but rather is implicated in many related dimensions (Dawe & Loxton, 2004). Reduced 5-HT transporter levels in the PFC have been found to be associated with heightened impulsivity, cognitive impairments, and anxiety-like behaviour (Lesch & Merschdorf, 2000). This is supported by an animal model that uses mice lacking the 5-HT transporter (5-HTT). Across a battery of behavioural tests for anxiety-like behaviours (elevated plus maze, light/dark box, emergence test and OF test) the male and female mice exhibited increased anxiety-like behaviour relative to mice with normal 5-HTT functioning. The administration of 5-HT receptor antagonist reversed the anxiety-like behaviour displayed by the mice lacking 5-HTT. Holmes, et al. (2003) concludes that abnormalities in 5-HTT levels contributes to anxiety-like behaviours and is associated with emotional disorders, including sensitivity to DOA (Holmes, Yang, Lesch, Crawley, & Murphy, 2003). It has also been suggested that 5-HT depletion in animals increases impulsivity by possibly removing the inhibitory effects of 5-HT on dopamine transmission (Nordahl, et al., 2003). Therefore, the binge-treated animals may have suffered greater 5-HT or 5-HTT damage, inducing heightened impulsivity compared with the distributed group. The issue of impulsivity needs to be further addressed, as impulsivity is implicated in drug abuse (Jentsch & Taylor, 1999). An alternative explanation may be that the binge animals were motivated by a desire to escape from the apparatus itself (Roy & Chapillon, 2004). Both possible explanations for this difference need to be further examined. Nevertheless, this study suggests that BZP exposure on adolescent rats in larger quantities over a shorter period of time may result in significantly more anxiety-like behaviour in adulthood than a smaller quantity of BZP administered over a longer time frame.

Control rats, compared with the two groups of treated rats, reared more in the middle unprotected squares of the OF. Rearing behaviour is implicated in emotionality. That is, decreases in rearing behaviour are thought to indicate higher anxiety (File & Seth, 2003). This finding is in accord with the other two measures of emotionality. BZP-treated animals show higher levels of anxiety-like

behaviour in adulthood. Although effects of BZP exposure on rearing in the outside protected squares of the OF were not significant in this study, the results are suggestive in that BZP-exposed rats made fewer rears than control animals ($p < 0.067$). Possibly, with higher numbers of animals, this difference would have been significant. Nevertheless, the trend was consistent with other findings.

Research suggests that lower levels of interaction in the social interaction test indicate higher levels of anxiety (File & Seth, 2003). The control animals, compared with the two BZP-exposed groups, spent significantly longer periods of time in social interaction with an unfamiliar conspecific. As the social interaction test measures social anxiety (File & Seth, 2003), BZP-treated animals may experience increased vulnerability to experiencing social anxiety, in addition to possible increases in the equivalence of GAD. This is important as social anxiety is thought to lead to increases in mood and anxiety disorders in humans (Palanza, 2001).

It is clear that for most behavioural measures, there was a significant difference between the control and the two BZP-treated groups. In addition, the administration of a larger quantity of BZP in adolescence over a shorter time frame significantly affects anxiety-like behaviour in adulthood, compared with a smaller quantity of BZP over a longer time frame. Therefore, the primary aim of this study was achieved and additionally, the question of what dosing regime is more detrimental may have been answered.

6.1.1 Sex Differences

A significant sex difference was observed in total entries in the Y-maze. In the Y-maze female rats made significantly more entries of both arms than the male rats. This is consistent with most research using both male and female rats: female rats generally are more active than males (Bridges & Starkey, 2004; Hughes & Neeson, 2003; Palanza, 2001; Ramos, Berton, Mormede, & Chaouliff, 2002). Alternatively, there is also research that suggests that only after puberty, do male rats

decrease their activity compared with females (Garau, et al., 2000). However, there was one difference between males and female in responsiveness to BZP treatment reflected in the sex x group interaction in total time in both arms of the Y-maze. The control male rats spent more time in both arms than the BZP-treated males and all three groups of female rats. An explanation for this finding is that, because adolescent male rats produce more DA and subsequent reduction of this neurotransmitter compared with female rats (McFadyen, et al., 2002); the exposure to BZP may have altered the DA levels more drastically for the male animals. Therefore, the administration of BZP to the still-developing brain in adolescence may result in male animals being more susceptible to the drug induced changes in neurotransmission, than the female animals. This result suggests that male treated rats compared with male controls are more affected by BZP when exploring both arms of the Y-maze.

Females, emerged significantly faster than males from the light/dark box and displayed significantly higher active exploration in the OF. This result is consistent with the finding from the Y-maze and was supported by significantly more time in active exploratory behaviour by female animals in the social interaction test. Additionally, in the social interaction test, male animals spent significantly longer time in active social interaction than female animals. However, this is possibly accounted for by the significantly higher locomotion in the females compared with the males. It is probable that, as the females spent more time in active exploration of the maze, they subsequently spent less time in social interaction. This supports the view that male animals may be primarily motivated in behavioural tests by sexual encounters and anxiety, whereas female rats are motivated by activity and exploration behaviours (Fernandes, Gonzalez, Wilson, & File, 1999). In a factor analysis studying the behaviour of male and female rats on a variety of behavioural tests, Fernandes, et al. (1999), concluded that anxiety was one of the main factors that accounts for male rat behaviour, whereas activity is a predominant factor for females behaviour. Therefore, there may be an innate difference between male and female animals and the present results found may just be reflecting

these inherent sex differences regardless of the treatment given.

The male animals in the present study spent longer in social interaction but also defecated significantly more often than females, thereby failing to support the findings of File and Seth (2003), who state that social interactions are negatively correlated with higher frequencies of freezing behaviour or defecation. Yet, File and Seth (2003) address sex differences in general and not long-term outcomes of drug-exposed behavioural changes. Nevertheless, in this study the male rats also significantly defecated more often than females in the light/dark box test, which supports the similar results of the social interaction test. Male rats in general may defecate more than female rats supporting File and Seth (2003), but the fact that the male animals in this study also spent more time in interaction than the female animals fails to support File and Seth (2003). However, the control animals spent significantly longer in social interaction than the two BZP-treated groups, which suggests that the exposure to BZP in adolescence has contributed to the inconsistent findings.

6.2 Methodological Limitations

The strengths and the weaknesses of the study may determine the extent to which the findings reported can be generalized. For example, the drug was administered by the experimenter rather than through a self administration regime. This needs to be further investigated because the neurobiological effects of BZP may vary depending on whether the animal chooses to take the drug or not (Robinson & Kolb, 2004). As mentioned above, there is a difference between an animal seeking the drug (Andersen, et al., 2002) and actually being given the drug (Brandon, et al., 2001). However, in some brain regions both self administration and experimenter administration produce the same changes. For example, AMP administered either way increases the spine density in the NAc and the PFC (Robinson & Kolb, 2004), therefore, affects behaviour in a similar manner.

Another concern is the administration of BZP by way of intraperitoneal (i.p.) injection. Human

users of BZP typically take the doses orally. Voluntary oral self-administration of BZP, as with other DOA, would be the optimal route of administration (Carlezon & Konradi, 2004), but there is no research to suggest that rats will orally take BZP. However, studies indicate that there is no protective effect of oral, as opposed to other routes of administration, and the effects of i.p. administration of AMP are similar to the effects of oral administration in rats (MacCann & Ricutre, 2004). Moreover, i.p. administration is a relatively slow and gradual process similar to oral administration (Blanchard & Blanchard, 1999). It must be stressed that all groups had the i.p. exposure to either saline or BZP and therefore the results found cannot be attributed to differences in experimental procedure, but rather are due to BZP itself. Therefore, regardless of route of administration, the long-term behavioural effects are believed to be the same.

The results of this study indicate that increased anxiety-like behaviour in adult rats followed BZP exposure during adolescence. The behavioural measures used for this study utilised the natural approach/avoidance conflict that rats display (File & Seth, 2003). That is, a natural conflict between curiosity about a novel environment and avoidance of a potentially threatening environment. However, whether or not this type of conflict is relevant to humans is questionable. One study that throws some light on this issue is that of Blanchard et al. (2001), in which 161 male and female undergraduate students were interviewed. The participants read a set of 12 scenarios based on defensive responding in rodents (e.g. magnitude of threat, the need to escape the situation, distance between the threat and the subject, ambiguity of the threat stimulus and the presence of a hiding place). The authors concluded that the patterns of defensive behaviour and emotional responding are similar for rodents and humans (Blanchard, Hynd, Minke, Minemoto, & Blanchard, 2001) with no significant differences between males and females. That is, humans (both males and females) will react in a similar way to rats to a present or potentially threatening conspecific or environment.

There was also a potential confounding factor in regard to the dosing procedure used in this study.

One group of animals was given what was considered to be a binge dosing regime, and the results suggested that these animals displayed higher levels of anxiety-like behaviour in adulthood than the distributed animals. But, for the first 4 days of BZP administration, the distributed group received 10 mg/kg/day, and the binge group received 25 mg/kg/day. It is therefore possible that, even if the distributed group had received no more BZP after these 4 days, differences between the two types of treatment might still have proved to be the same as reported. In other words, the results might have merely reflected dose-response effects of the 4 treatment days, rather than differences between the same total quantity of BZP experienced over two differing time frames i.e., four days of 25 mg/kg/day versus 10 days of 10 mg/kg/day. This needs to be further investigated before any firm conclusions can be drawn regarding dosing regimes. Nonetheless, irrespective of the exact reasons, binge-treated animals appeared to display higher amounts of anxiety-like behaviour than the distributed animals.

6.3 Methodological Strengths

An advantage of this study in measuring behaviour is that, because rats develop rapidly; the two weeks between the last exposure to BZP and rats reaching adulthood is sufficient time to ensure that there is no drug still affecting their performance (Snyder, Katovic, & Spear, 1998). In addition, the increased anxiety that was displayed from the two treated groups was unlikely to be due to the handling, weighing or experimental procedures because the control animals were treated in exactly the same way.

Although direct extrapolation of findings from the present research to humans must be approached cautiously, many former animal models have been useful in predicting long-term responses in humans. For example, Andersen et al (2002) showed that chronic treatment with typical neuroleptic medications can induce brain morphological changes that are similar in rodents and humans (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002). One study of the metabolism of BZP in male Wistar rats found that the same identified metabolites were found in the rats' urine as

was found in human urine (Staack, & Maurer, 2005). Therefore, the results gained from this study may be applicable to a human population, bearing in mind that they are for BZP alone and do not take account of other drugs that human users might take in addition to BZP.

Human users may often self-administer other drugs in addition to party pills. In many human studies there is little control over the amount of drug used, the purity of it and any polydrug habits of the subjects. As BZP is the main base drug in most party pills the present research provides a starting point for further research involving different drug combinations. Therefore, this study addressed the long-term effects of BZP in a way that avoids the complex issues of polydrug use, drug purity and any pre-drug psychopathology that can possibly compromise human drug research (Koenig, et al., 2005). The animal model used in this study controls for the history, experiences and environmental conditions of the subject. As it is usually impossible to control for all of these in humans, this study provides an example of relatively uncontaminated BZP effects.

Consequently, this protocol was not intended to exactly replicate human consumption situations; rather it was intended to capture some of the aspects of regular dosing of BZP over a longer interval and higher dosing over a shorter interval. In this way, it serves as a valuable starting point for future research. As to date there have been no studies of the long-term effects of BZP exposure, such effects of BZP can only be inferred from results with comparable drugs. But a major problem in this respect is the lack of consistent findings with these comparable drugs.

6.4 Consistent and Inconsistent Findings

Because this is the first long-term study of the effects of BZP exposure on the developing brain, mechanisms can only be inferred from the effects of other stimulant drugs. Long-term increases in anxiety have been found with AMP (Cancela, Basso, Martijena, Capriles, & Molinal, 2001), METH (London, et al., 2004; Rawson, et al., 2002), MPH (Balanos, 2003; Carlezon, et al., 2003), and MDMA (Clemens, et al., 2004; Gurtman, et al., 2002; Ho, et al., 2004; McGregor, et al., 2003). Rats

exposed to AMP show a greater avoidance of the two exposed arms of an Elevated plus maze (which are probably comparable to the novel arm of the Y-maze). Similarly, METH administered to rats has been shown to later decrease social interaction and longer emergence time from the light/dark box, whereas MDMA has been shown to decrease social interaction but not emergence time (Clemens, et al., 2004).

Conversely, Piper and Meyer (2004) found that MDMA treatment given to adolescent rats decreased anxiety-like behaviour in adulthood. The MDMA-treated animals showed increased amount of time spent on the open-arms of the Elevated plus maze. However, as the rats in Pier and Meyer's study were administered MDMA from PND35 to PND60, their finding is possibly consistent with childhood administration of MPH, in that, earlier dosing acts to some extent as a protective factor (Carlezon, et al., 2003). Therefore, discrepancies between Piper and Meyer (2004) and those of Clemens, et al. (2004), may be accounted for by age of dosing and the behavioural measures used. In contrast, other studies have found elevated levels of anxiety shown as a longer delay before emerging from the light/dark box, four weeks after MDMA treatment. Six weeks after MDMA treatment, rats have exhibited higher anxiety in the social interaction test (Gurtman, et al., 2002). Eight weeks after administration MDMA, increased anxiety as reflected in both social interaction and light/dark box tests (McGregor, et al., 2003), and after nine weeks increases in anxiety were observed in the Elevated plus maze (Gurtman, et al., 2002). The results of this study were consistent with those for other stimulants, namely increased anxiety from exposure to BZP in adolescence.

Contrary to the above described results are studies of adolescent alcohol use. Salimov et al. (1996) gave adolescent rats alcohol and tested them in adulthood and found that the treated rats had reduced novelty-induced anxiety. However, they also spent more time immobile (Salimov, McBride, McKinzie, Lumeng, & Li, 1996). This might be explained by the fact that alcohol is a CNS depressant, whereas, BZP and the other drugs mentioned above are stimulants.

Benzylpiperazine undoubtedly has CNS stimulant properties, and the results found in the present study are consistent with other long-term findings after administration of stimulant drugs. Stimulant drugs increase the amount of DA and 5-HT in the synapses by decreasing the reuptake of these neurotransmitters. Animals that have 5-HT damage or are under the influence of drugs that impair the 5-HT system, experience more anxiety than controls (Graeff, Viana, & Mora, 1997), consistent with the results found in this study. In summary, a long-term consequence of the use of stimulant drugs including BZP in adolescence is increased anxiety in adulthood, because of as yet undetermined changes in the brain's functioning.

7.0 General Discussion

The results of this study supported the primary aims. Rats exposed to BZP in adolescence displayed significantly higher anxiety-like behaviour in adulthood, compared with control rats. Additionally, the adolescent animals exposed to the binge dose regime, exhibited elevated emotionality in adulthood compared with adolescent animals exposed to daily BZP administration.

7.1 Neurodevelopment

Findings of this study support previous long-term effects found with other stimulant drugs, and BZP has been shown to have stimulant properties (Baumann, et al., 2005; Bishop, et al., 2005; Campbell, et al., 1973; De Boer, et al., 2001; Fantegrossi, et al., 2005; Peters, et al., 2003; Staack & Maurer, 2005; & Wikstrom, et al., 2004). For example, Melega, et al. (1998) have established, using (PET) scans of monkeys brains, that after ten days of METH use DA production is significantly reduced for an entire year. Full recovery is not seen until two years later (Melega, et al, 1998). Damage to the DA nerve terminals in animals exposed to METH is similar to damage observed in human METH users (Nordahl, et al., 2003). Conversely, there is no evidence to suggest that DA alone is

responsible for the continued use of drugs, but as White and Kalivas (1998) state, something else is contributing to the alterations in DA levels. File and Seth (2003) found that an 80% depletion of 5-HT in a rat's amygdala results in significantly less social interaction in the social interaction test and reduced locomotor activity. Similar outcomes have been documented in human AMP users (Nordahl, et al, 2003) and this is proposed to be from altered 5-HT modulation of the ventral PFC and its interrelated structures causing impaired judgement and increased impulsivity. Baumann, et al. (2005) suggest that BZP affects extracellular DA and that the increase in DA transmission leads to behavioural effects similar to those of AMP. However, they also state that the long-term behavioural effects of BZP are yet to be explained, and any contributions of 5-HT to the behavioural effects have not yet been reported. Evidence from the present study suggests that long-term increases in emotionality may be due to drug effects on the 5-HT system, as implicated by increases in anxiety-like behaviours expressed in adulthood by BZP-treated rats.

This research further expands recent findings in the area of 5-HT transmission. Investigations are only beginning to clarify how 5-HT is affected by DOA. There is evidence that AMP produces long lasting changes in the DA and 5-HT systems in many mammals (McCann & Ricaurte, 2004). Similarly, MDMA produces long-term damage to 5-HT neurons (Bauman, et al., 2005; Green, et al., 2004), and the damage to the 5-HT system seen in animals, is similar to doses frequently used by humans (Ricaurte & McCann, 2005). Additionally, animal research has implicated 5-HT deficits in heightened impulsivity (Lesch, & Merschdorf, 2000). The results of this thesis suggest exposure to BZP in adolescence may have affected the still developing 5-HT system.

The results of this study suggest that alterations to the 5-HT and/or 5-HTT system while still developing, invoked increased anxiety-like behaviour and may have also increased impulsivity. Impulsivity typically refers to behaviours that include a lack of foresight or planning, rashness or behaviour that occur without careful deliberation. Generally, impulsivity describes behaviours that

happen without consideration for the consequences of that behaviour (Dawe & Loxton, 2004). Neuroimaging technology has implicated impairment in the PFC as contributing to disinhibited behaviour (impulsivity) and the inability to discontinue substance use despite negative consequences (Jentsch & Taylor, 1999). Impulsivity is implicated in substance misuse (Dawe & Loxton, 2004) and BZP-exposed animals in this study may have had altered 5-HT functioning, which in turn was linked to impulsivity, leading to a possible heightened vulnerability to the development of SUDs.

Decreased social interaction with novel unfamiliar pairs of rats was observed in both treated groups in the social interaction test, which suggests that earlier BZP exposure produced an increased anxiety-type response to the interaction. This is similar to the research into the long-term effects of MDMA. Morley et al, (2001) found decreased social interaction in the social interaction test and longer emergence times in the light/dark box in animals treated with MDMA and tested three months after the initial administration. They concluded that MDMA caused neural damage, possibly in the brain 5-HT system. Importantly, the results from Morley et al, (2001) were found in adult animals, suggesting that even once the brain is fully developed neuronal imprinting can still occur. This supports the possibility of the 5-HT systems being affected by adolescent BZP exposure, because, 5-HT is implicated in regulation of emotion. Moreover, this and other neurotransmission circuits are not fully developed in adolescence and importantly the long-term increased anxiety displayed by MDMA-exposed rats was found in adult-dosed animals (Morley, et al., 2001). Because the animals used in the present study were administered BZP in adolescence, it is possible that the drug interfered with 5-HT development during this phase. For example, increases in 5-HT activity induced by adolescent exposure to BZP, altered the normal functioning of the 5-HT and 5-HTT system that may have resulted in decreased 5-HT and 5-HTT activation in adulthood, which is one explanation for increases in anxiety-like behaviour (Holmes, et al., 2003).

There is mounting evidence that stimulant drugs affect not only the brain's DA system but also the

5-HT system, as discussed earlier in section 1.4. As the brain, primarily the PFC, is not fully developed in adolescence, any changes induced by BZP to the 5-HT system may account for increases in emotionality observed in this study. Modification of serotonergic mechanisms has additionally been implicated in impulsivity. This raises an important issue because impulsivity and executive functioning appear to be interrelated and impulsivity is proposed to be a risk factor for drug use (Rogers, et al., 1999). If the premise of this study is correct, then the BZP-exposed animals may have had reduced 5-HT in the PFC because of earlier BZP exposure. In addition, to increases in anxiety, this reduction of 5-HT could in turn, lead to either individuals self-medicating with illegal drugs because of negative affect, or conversely, using drugs because of greater impulsivity or risk-taking behaviours. The results from the light/dark box suggest that binge-dosed animals may have had increased impulsivity in addition to increased anxiety probably arising from 5-HT alterations to the PFC.

7.2 Neuronal Imprinting

The results of this study support the neuronal imprinting theory, that is, the effects of the drug on the developing nervous system can be observed after a period of abstinence from the drug. This research suggests that the mechanisms that control mood, and responses to stress have provoked a gradual shift to a new “stasis” (set point), which in pathological cases, would be clinically defined anxiety disorder (Millan, 2003; Stanwood & Levitt, 2004). It is clear that neuronal imprinting occurred in this study. However, exactly where in the brain this occurred is unknown. One suggestion, is that BZP increased 5-HT levels in the brain in adolescence, causing inactivation of the 5-HT system while it was fully maturing, thus leading to decreased amounts of 5-HT produced in the brain in adulthood. The fact that adolescence is associated with more risk-taking behaviour and adolescents are using so-called “legal highs” before the brain is fully developed, is concerning. It is suggested, that adolescent substance abuse is associated with later mental health problems (Carlezon & Konradi, 2004), cognitive impairment (Brook, et al., 2002), behavioural change and health and

physical changes (Aarons, et al., 1999; Brook, et al., 2002; Laviola, et al., 1999; Smith, 2003). The support established by this research for the neuronal imprinting theory is vitally important because of the long-term effects on the present generation of adolescents when they reach adulthood. Census data indicates that adolescents and young adults (15- 24 years of age) make up 15 to 16 percent of the total population (Ministry of Youth Affairs, 1996). In five years or so it is possible that a significant section of the entire population may be experiencing the detrimental effects of what is considered presently to be a safe and legal drug.

7.3 Temporal Ordering

The results of this study support the theory that substance use precedes psychiatric disorders. This must be approached with caution however as the animals used in this study were ‘normal’ animals without the equivalent of underlying depressive or mood disturbances experienced by humans. Therefore, the results of this investigation primarily addresses the question of how BZP may effect the normal brain and further studies need to address the consequences of earlier BZP exposure on animals with an underlying pathology. Additionally, this study was a study of groups rather than individuals and there will be differences between individuals, possibly, because of variations among the many psychological, environmental and biological factors as mentioned in section 1.2. However, the results support the idea that substance use can precede anxiety, but as the self-medication hypothesis was not tested in this study, any statements regarding causality or temporal ordering must be approached with extreme caution.

Earlier-onset mood disorders generally begin in adolescence, while subsequent substance disorders do not usually emerge prior to young adulthood (Brook, et al., 1998). Both psychiatric disorders and substance use however are believed to share the same common etiological factors. For example, predisposing genetic or biological factors may be attributed to disorders of neurotransmitter processes or metabolism (Brook et al., 1998). This is extremely important if the individual seeks

treatment for either the substance abuse or the anxiety, as individuals with comorbid disorders have poorer treatment outcomes (Tomlinson et al., 2006). The testing of the self medication hypothesis also needs to be explored with BZP. Adolescents with underlying anxiety may take the drug initially as part of New Zealand youth culture and subsequently find that BZP may reduce or eliminate unpleasant feelings. Therefore, an individual, possibly with an existing mood disturbance, may continue to take the drug because of its rewarding effects.

7.4 Implications for Adolescence

The implications of this study for the greater population may be far-reaching, regardless of whether or not researchers fully agree about the validity of animal models in studying drug-induced anxiety. The results support temporal ordering (i.e., substance abuse can precede mood disorders) and neuronal imprinting (i.e., effects of the drug are long-term) theories. BZP may interfere with the brain's ability to reorganize neural circuits and earlier drug exposure could interfere with the cognitive advances that are made through development (Robinson & Kolb, 2004).

This is critical as alterations in cognitive functioning may interfere with quality of life (Carlezon & Konradi, 2004), possibly because of difficulties in social cognition and decision-making (Roger, et al., 1999). For example, a clinical investigation comparing 18 AMP and 13 opiate abusers, with 20 patients with PFC damage and 26 controls (matched for age and intelligence). They were assessed on decision-making behaviour. The results found included increased deliberation times to make a choice in the AMP, opiate and PFC damaged patients and poorer quality of decision-making by PFC damaged patients and AMP abusers. For the AMP abusers this was not attributed to increases in deliberation times, but rather correlated negatively to the number of years of abuse. Interestingly, tryptophan depletion in the control group produced the same outcomes as shown by the AMP and PFC groups. Because tryptophan depletion reduces central 5-HT activity, the authors concluded that AMP abusers and patients with damage to the PFC were similar, possibly because of deficits in the

neuromodulation of 5-HT and its inter-connected systems (Rogers, et al., 1999).

Anxiety and cognition interact in a fundamental way, and several studies suggest that cognitive dysfunctions may be the primary presenting feature of anxiety (Ohl, 2003). There is also considerable research that suggests that the mechanisms that are involved in emotion are also involved in learning and memory (Conrad, et al., 2004; Wolf, Sun, Mangiavacchi, & Chao, 2004). As the results of this study suggest an increase in anxiety, the interrelationship between anxiety, cognition, learning and memory may lead to BZP users experiencing more seriously adverse outcomes because the adolescent brain is not fully developed. Vorhees et al. (2005), found that rats which were given METH from PND41-50 had deficits in spatial learning/reference memory and sequential learning. The rats administered METH before PND41 or after PND50 did not show the same deficits (Vorhees, et al., 2005). This supports the premise that the adolescent brain is undergoing important changes during this time and the administration of a drug can cause long-lasting persistent behaviour change. Although, memory and learning were not measured directly in this study, it may be acceptable to point out that DA and 5-HT are involved in learning, memory and emotion. Therefore the findings of Vorhees and co-authors may also be applicable to BZP-exposed animals and relevant to BZP users who consume higher quantities of the drug over a shorter time frame.

Notwithstanding the possible confounding of binge effects by dose-response effects, there may be more negative implications for individuals ingesting higher quantities of BZP over a shorter time frame, than a smaller quantity over a longer time frame. In addition to the increased anxiety-like behaviour displayed across all behavioural measures, the social interaction test is proposed to measure social anxiety (File & Seth, 2003). In the social interaction test, decreased interaction time is comparable to the well-documented occurrence of paranoia, anxiety and social withdrawal in human METH users (Rawson, et al., 2002). In addition, paranoid-like psychosis after long-term

stimulant use is well known and has been attributed to binge use (Segal & Kuczenski, 1997). Although, the binge-treated rats in this study exhibited significantly more emotionality than the daily dosed rats, it is difficult to relate this to human stimulant psychoses. In spite of this, the implications for adolescents taking a greater quantity of BZP over a shorter time period appear to be worse for adulthood functioning than a smaller quantity over a longer time frame.

7.5 Stage Theory of Addiction

Although this investigation did not address the stage theory of addiction, it is too important not to be mentioned. The stage theory of substance use believes that the development of substance use and or abuse follows a likely progression from legal drugs to illegal drug use (Smith, 2003). BZP is legal in New Zealand and because research indicates that there is a natural progression from legal drugs to illegal drugs (MacCoun, 1998), BZP may be included with other gateway drugs. Admittedly, not all individuals will experiment as adolescents and consequently meet the DSM-IV criteria for SUD in later adulthood (Crombag & Robinson, 2004). It must be stressed however that support for the stage theory of addiction (Fergusson & Horwood, 2000; Smith, 2003; Walker, et al., 2004) leads to important considerations that must be examined when evaluating the consumption of herbal highs in New Zealand. The heightened vulnerability to the addictive properties of drugs in adolescence is one reason for unease. BZP may have addictive properties (Fantegrossi, et al., 2005), yet it is marketed as herbal without informing the public that, even though legal they may not be different from any other DOA. It is the nature of young people to experiment with adult behaviours and this is to be expected in their search for adulthood identities. However, there is a current misconception in New Zealand namely, that there is no harm involved in taking herbal highs. It is considered better for adolescents to experiment with herbal highs instead of illegal drugs. However, the results of the research reported in this thesis suggest that this may not be the case.

Because of predictions based on the stage theory of addiction, BZP-based substances may now have to be viewed as possibly causing progression to illegal drugs. What happens if the individual

experiences tolerance to BZP? Will this lead to further usage with illegal stimulants to produce the initial effects? The nature and scope of this investigation provide a much needed starting point that to date has been lacking. Addiction is not merely a consequence of drug use. Instead, there are possible environmental factors and complex drug interactions that determine the likelihood of a drug producing alterations in the neuronal circuitry contributing to addiction (Crombag & Robinson, 2004). One of the leading risk factors into later drug use is the availability of drugs in an individual environment (Ellickson & Morton, 1999). It goes without saying how easily available BZP is for individuals in New Zealand.

8.0 Future Directions

This study highlights some important facts that will require additional research. Firstly, the stage theory of substance use in that there is a natural progression from legal drugs to illegal drugs, needs to be considered. Will the consumption of BZP in adolescence predispose the individual to later illegal drug experimentation? In other words, should BZP be considered a gateway drug? The neuronal imprinting theory was supported in this study, suggesting that early exposure to BZP leads to subsequent changes in adult behaviour. However, the alternative self medication hypothesis was not examined. To fully examine the long-term effect of the administration of BZP in adolescence, investigations are needed with individuals who experience underlying mood disorders and who are possibly self medicating with substances to alleviate negative emotions they might be experiencing. Finally, the ordering of dual diagnosis or temporal ordering of what precedes what, needs to be addressed. This study supports the theory that substance use precedes psychiatric disorder. However, the animals used were not genetically bred for underlying mood disorders, so testing for the possibility that a mood disorder leads to self medication was not possible.

Aggressive behaviour by the BZP-treated rats was observed but not measured in the social interaction test. Aggression was not measured because this study was the first to consider the effects

of BZP and no other research implicated aggression as a behavioural outcome. Aggression, impulsivity and risk-taking are all related to 5-HT depletion in the PFC and interrelated systems (Dawe & Loxton, 2004; Wills, et al., 1983). The aggression casually observed in the social interaction test and possible increased impulsivity displayed by the binge-treated animals in the light/dark emergence test suggests that, in addition to increases in anxiety, the BZP-treated rats may exhibit these other behaviours. This is consistent with the premise that BZP caused an alteration in 5-HT activity. However, this needs to be further addressed, as aggression, impulsivity and risk-taking are also correlated with substance abuse (Chambers, et al., 2003). Notwithstanding, increased anxiety leading some individuals to self-medicate with substances, the other behaviours mentioned may additionally increase receptiveness to experimentation with illegal drugs.

BZP was introduced into New Zealand under the harm reduction umbrella, yet there is no evidence to support this premise. This study might serve as a start for additional research into this assumption. “Agonist substitution” therapy involves administering substances that are less potent and less addictive than the DOA. This can be described as “normalisation” therapy, in which the administration of a less potent substance normalises deregulated neurochemistry provoked by the DOA (Rothman & Baumann, 2003). The possibility that individuals will substitute AMP/METH for BZP needs to be further explored. Since Hashimoto et al. (1992) suggest that BZP inhibits the reuptake of 5-HT which has been implicated in the rewarding properties of stimulant-like drugs, normalization therapy may work as a substitute for AMP/METH-dependent individuals.

There is growing clinical evidence that the period of adolescence is a period of heightened vulnerability to the addictive properties of both legal and illegal drugs (Chambers, et al., 2003). The results of this study support the idea of vulnerability of the adolescent brain and, further research needs to continue to utilise adolescent male and female animals to answer questions that this research has raised. Firstly, will adolescent BZP-exposed animals subsequently seek other drugs, to

examine the stage theory of addiction? This would imply that BZP may act as a gateway drug for further experimentation. Secondly, will animals with underlying initial anxiety self-administer BZP to alleviate negative emotions? This would address the self-medication hypothesis. Thirdly, the two behaviours observed but not measured were impulsivity and aggression. Both may be implicated in modifications of normal 5-HT functioning. Fourthly, will AMP/METH dependent animals self-administer BZP as a substitute for dependence on other stimulants? This would test the assumption that BZP can act as a replacement drug for AMP/METH dependence, thereby, supporting normalization therapy. Finally the lack of neurochemical analyses in this study needs to be addressed. This would help clarify whether or not the increased emotionality displayed by the animals in this research was due to 5-HT or 5-HTT modification in the brain, especially the undeveloped PFC.

9.0 Conclusion

Substance use is a reality (Resnicow, Smith, Harrison, & Drucker, 1999) and no amount of research into the detrimental effects of drugs can prevent this actuality. However, this study opens the way for future understanding of the undesirable effects that could be observed in the next generation of individuals reaching adulthood. The administration of BZP during this period may have caused a functional change in DA and 5-HT levels by altering modulation of the neurotransmitter release and/or altering the functional development of the PFC input, thereby leading to an increased risk of higher anxiety during adulthood. This higher anxiety during adulthood may lead to self-medication with illegal drugs, or worse still, dependence on these substances. Therefore, these ontogenetic changes, along with the fact that adolescence is the period of development when drug use is initiated, provide a compelling reason for future research into the effects of BZP.

The main limitation of the present study is clearly the lack of neurochemical analyses of the brains of the rats exposed daily to or binge-administered BZP during adolescence. While it is hypothesised

that the observed effects on anxiety relate to the neurotoxic action of BZP on the brain's 5-HT systems, conclusive proof of this is lacking. Notwithstanding the lack of neurochemical analyses, and possible confounding of binge effects by dose-response effects, the results of the present study are strikingly clear. Rats given BZP in adolescence show increased anxiety-like behaviours in adulthood compared with untreated controls.

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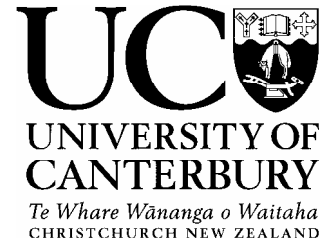
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Appendix 1



Ref: 2005/14R

2 May 2005

Lara Aitchison
Department of Psychology
UNIVERSITY OF CANTERBURY

Dear Lara

I am pleased to inform you that the Animal Ethics Committee has approved your application entitled: **Adolescent benzyloperazine (BZP) exposure in rats: adulthood changes in memory and emotion.**

Yours sincerely

Dr Lou Reinisch
Dean of Science